Carbon and Nitrogen Removal at a Full-Scale Municipal Drinking Water Treatment Plant employing Sand-ballasted Clarification, Ozone and Biofiltration

by

Lizanne Pharand

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Lizanne Pharand

Abstract

Natural organic matter (NOM) is composed of a complex matrix of organic compounds originating primarily from plant and animal degradation products, including both carbon and nitrogen, and is found in all natural waters. The removal of NOM in drinking water treatment plants is of importance as its presence is associated with qualities responsible for adverse aesthetic concerns such as colour, taste, and odour. It can also substantially impact treatment processes, as it has been shown to increase coagulant and disinfectant demand, corrosion and bacterial regrowth in distribution systems, and interfere with adsorption processes. More critically, certain NOM fractions have been identified as being precursors to potentially harmful disinfection by-products (DBPs) which over time can cause a variety of cancers in humans.

The goal of this research was to determine the removal of carbon and nitrogen NOM components through a full-scale municipal drinking water treatment plant employing advanced treatment strategies aimed at reducing NOM, including sand-ballasted clarification (SBC), ozonation, and biological filtration (biofiltration). Investigation into the effect of seasonal changes in raw water quality and temperature on process performance, and determination of biofilter biomass quantity and activity were also carried out. The approach used to accomplish these goals involved sampling water and biofilter media from the Holmedale Water Treatment Plant (HWTP), located in Brantford, Ontario over a period of 14 consecutive months.

NOM components were identified using a recently developed NOM characterization technique, liquid chromatography-organic carbon detection (LC-OCD), which fractionates NOM based on size and provides information about the concentration of five operationally defined NOM fractions. The fractions include biopolymers, humic substances, building blocks, low molecular weight (LMW) acids & humics, and LMW neutrals. The carbon fraction of NOM was quantified further using traditional water quality indicators, such as total organic carbon (TOC), dissolved organic carbon (DOC), assimilable organic carbon (AOC), ultraviolet absorbance at 254 nm (UVA_{254}), and specific ultraviolet absorbance (SUVA). The nitrogen fraction of NOM was primarily investigated by quantification of inorganic nitrogen forms, such as total nitrogen, nitrate and ammonia.

Throughout the sampling campaign, considerable removal of carbon compounds through sand-ballasted clarification was observed. Ozonation led to a substantial increase in AOC, which was anticipated (and for the most part removed through downstream biofiltration). The performance of

both sand-ballasted clarification and ozone did not change considerably with seasonal temperature changes. The biofilters were capable of considerable removal of most carbon containing compounds, although the removal of certain fractions, suspected as being biodegradable, was reduced at cold raw water temperatures. Somewhat unexpectedly, no removal of total nitrogen, nitrate, or ammonia was observed through SBC, ozonation, and/or biofiltration.

Due to the limited number of peer-reviewed articles on full-scale biofilter biomass characterization, investigation into the biomass quantity, as determined by adenosine triphosphate (ATP), and biomass activity, as determined by fluorescein diacetate (FDA) hydrolysis was undertaken. A review of the available literature demonstrated that the ATP concentration at the surface of active, acclimated biofilters (with granular activated carbon [GAC] or anthracite media) is typically in the order of 10^2-10^3 ng ATP/cm^3 media. Compared to this benchmark, the biofilters at the HWTP appeared to contain a considerable quantity of active biomass. Nonetheless, results from the literature review and from this investigation demonstrate that no relationship exists between biofilter performance, in terms of organic matter removal, and ATP concentration at the surface of biofilters. Further investigation was also performed to determine if the biomass within the biofilters was receiving sufficient essential nutrients, namely carbon, nitrogen, and phosphorus, for growth. Determination of the carbon:nitrogen:phosphorus (CNP) ratio in the biofilter feed, and comparison to a widely accepted benchmark of roughly 100:10:1, suggested a potential phosphorus limitation. However, good biofilter performance, in terms of AOC removal, biomass quantity, and biomass activity was consistently observed. Still, no relationship between CNP ratio and biofilter performance, biomass quantity, and biomass activity could be identified. Somewhat unexpectedly, raw water temperature did not appear to impact the biomass quantity (ATP), activity (FDA), or the CNP ratio in the biofilter feed.

The results from this research provide valuable information to municipal drinking water treatment providers whose plants employ SBC, ozone, or biofiltration. For the HWTP, although seasonal changes in raw water led to decreased biofilter performance for some monitored parameters, overall NOM removal through the plant remained considerable throughout the year. These findings provide insight to municipalities and consultants as it pertains to treatment process selection during the design or upgrade of drinking water treatment plants.

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List of Acronyms

AOC: Assimilable organic carbon

ATP: Adenosine triphosphate

AWWA: American Water Works Association

BDOC: Biodegradable organic carbon

BOM: Biodegradable organic matter

CNP: Ratio of carbon:nitrogen:phosphorus

COD: Chemical oxygen demand

DBP: Disinfection by-product

DNA: Deoxyribonucleic acid

DOC: Dissolved organic carbon

DON: Dissolved organic nitrogen

DWTP: Drinking water treatment plant

EBCT: Empty bed contact time

EPS: Extracellular polymeric substances

ES: Effective size

FDA: Fluorescein diacetate

GAC: Granular activated carbon

HAA: Haloacetic acid

HRT: Hydraulic retention time

HWTP: Holmedale Water Treatment Plant

LC-OCD: Liquid chromatography – organic carbon detection

LDL: Lower than detection limit

LMW: Low molecular weight

LP: Low-pressure

MAC: Maximum acceptable concentrations

MCL: Maximum concentration level

MIB: 2-methylisoborneol

MLD: Mega liters per day

MP: Medium-pressure

MRL: Minimum reporting level

n: Number of samples/observations

N: Nitrogen

NDMA: N-nitrosodimethylamine

NH_4: Ammonia

NO_3: Nitrate

NOM: Natural organic matter

NPOC: Nonpurgeable organic carbon

NSERC: Natural Sciences and Engineering Research Council of Canada

O_3: Ozone

ORF: Ontario Research Fund

OCWA: Ontario Clean Water Agency

P: Phosphorus

PACl: Polyaluminum chloride

PO_4^{3-}: Orthophosphate

SBC: Sand-ballasted clarification

s.d.: Standard deviation

SG: Specific gravity

SUVA: Specific ultraviolet absorbance

T: Temperature

TDP: Total dissolved phosphorus

THM: Trihalomethanes

TTHM: Total trihalomethanes

TN: Total nitrogen

TOC: Total organic carbon

TP: Total phosphorus

USEPA: United States Environmental Protection Agency

UV: Ultraviolet

UVA_{254}: Ultraviolet light absorbance at a wavelength of 254 nm

Chapter 1
Introduction

1.1 Problem Statement

Natural organic matter (NOM) is comprised of a complex suite of organic compounds and can be found in all natural waters. The composition of NOM in water varies substantially from one water source to the next, and depends on the surrounding environment (Fabris *et al.*, 2008). NOM predominantly includes carbon, oxygen, nitrogen and hydrogen atoms, and can be fractionated into different groups, each with unique characteristics (Thurman, 1985). The removal of NOM through drinking water treatment plants (DWTPs) is of particular interest as it leads to higher coagulant demand, transport of metals and chemicals, corrosion and bacterial regrowth throughout distribution systems, and interference in adsorption processes (Urfer *et al.*, 1997; Jacangelo *et al.*, 1995). Additionally, certain NOM fractions have been identified as precursors to potentially harmful disinfection by-products (DBPs) (Singer, 1999). Due to the complex nature of NOM, its quantity is often determined using surrogate water quality parameters such as dissolved organic carbon (DOC) and assimilable organic carbon (AOC). However, characterization of specific NOM fractions requires more sophisticated methods, such as liquid chromatography-organic carbon detection (LC-OCD) (Huber *et al.*, 2011). As the main components of NOM, and due to their role as precursors to DBPs, the removal of carbon and nitrogen containing compounds are of particular interest in DWTPs. Investigation into the removal of carbon and nitrogen through treatment processes in DWTPs, utilizing general water quality parameters and advanced methods such as LC-OCD, can provide valuable information relating to overall NOM removal efficiency of treatment processes.

Traditionally, municipal water treatment processes consisted of coagulation, sedimentation, flocculation, filtration, and disinfection, and were able to produce safe drinking water while trying to address taste, odour and colour concerns. However, as knowledge of DBPs increased, alternative water treatment processes capable of reducing the concentration of NOM were sought. Numerous advanced treatment processes and strategies aimed at reducing NOM through DWTPs have been developed and some include sand-ballasted clarification, ozonation, and biofiltration. Although numerous studies at bench- and pilot-scale have evaluated the efficiency of these treatment processes in terms of NOM removal only a limited number of studies have evaluated the efficiency of such treatment processes at full-scale over varying seasonal temperature and water quality ranges and few, if any, have included the breadth of data which can be provided by LC-OCD. Full-scale studies are of

great importance, as they can be used to confirm pilot-scale results, and may provide insight into the design and upgrade of municipal DWTPs.

The use of biofiltration in North America, as an advanced treatment technology for NOM removal, has increased due to more stringent water quality regulations, and the increased use of ozone treatment (Urfer *et al.*, 1997). Ozonation has been shown to lead to an increase in biodegradable organic matter (BOM), which can cause regrowth within distribution systems. However, biofiltration following ozonation, can reduce easily BOM to low concentrations and has also been shown to lead to a reduction in DBP formation (Urfer *et al.*, 1997). Although several studies have investigated the use of biofiltration at pilot-scale, performance monitoring of biofilters at full-scale is not common practice. Full-scale biofilter performance data, in terms of carbon and nitrogen removal, are important to ensure optimized operation of biofilters. Additionally, investigation into the activity of the biomass within full-scale acclimated biofilters is critical in understanding biofilter performance. Monitoring of biomass quantity and activity can provide for a greater understanding of the relationship between biomass and performance, which at present, is not well understood. This investigation was made to address many of these informational needs.

1.2 Objectives

To investigate the performance, at full-scale, of sand ballasted clarification, ozonation, and biofiltration using an advanced NOM characterization technique, two major goals were identified for this research: (1) the quantification of carbon and nitrogen removal through an operating full-scale municipal DWTP employing sand-ballasted clarification, ozone, and biofiltration, and (2) investigation into full-scale biofilter performance and biomass activity.

To achieve the first goal, the following objectives were identified:

- Quantification of various carbon compounds, such as total organic carbon (TOC), DOC, NOM fractions, and AOC to gain a greater understanding of the removal of carbon through sand-ballasted clarification, ozone, and biofiltration.
- Quantification of various nitrogenous substances, including total nitrogen, nitrate and ammonia, to gain a greater understanding of the removal of nitrogen through sand-ballasted clarification, ozone, and biofiltration.

- Investigation into the effect of seasonal variations in water temperature and quality on carbon removal, nitrogen removal, and overall plant performance.

To achieve the second goal, the following objectives were identified:

- Determination of biofilter biomass growth through quantification of the amount of viable cells present within the biomass and the activity of the cells, with the use of adenosine triphosphate (ATP) and fluorescein diacetate (FDA) hydrolysis analyses, respectively.
- Investigation into nutrient availability in the biofilter feed water, through carbon, nitrogen, and phosphorus quantification.

1.3 Approach

A 14-month investigation into the performance of the full-scale treatment processes at the Holmedale Water Treatment Plant (HWTP) was undertaken from May 2012 to July 2013. The HWTP is located in Brantford, Ontario and included the following treatment processes at the time of the present study: sand-ballasted clarification, ozonation, biofiltration, ultraviolet (UV) disinfection, and chlorine disinfection. Over the course of the study, raw water characteristics were monitored to determine the impact of seasonal changes in water quality and temperature on full-scale performance of treatment processes. Unit process performance was quantified by carbon and nitrogen compound removal through determination of TOC, DOC, NOM fractions, AOC, total nitrogen, nitrate and ammonia. NOM fractions analyzed by LC-OCD included biopolymers, humic substances, building blocks, low molecular weight (LMW) acids and humics, and LMW neutrals (Huber *et al., 2011)*. Biomass characterization was undertaken to determine the quantity of viable cells present within the biomass and the activity of the cells, through ATP and FDA hydrolysis analyses.

1.4 Thesis Structure

Chapter 2 includes a literature review providing an overview of published information related to this work, and describes each process at the HWTP. Certain processes introduced in Chapter 2 were included for completeness, although they were not discussed as part of this investigation. Each subsequent chapter was written in the form of a journal article, and each includes a dedicated methods section as well as results and conclusions (i.e. a paper-format thesis). Chapter 3 introduces the ATP and FDA hydrolysis methods for quantification of viable biomass/activity within biofilters. This chapter includes previously published information from the literature compiled for comparative

purposes. Chapter 4 provides information related to NOM removal through sand-ballasted clarification. Chapter 5 presents performance data for the full-scale pre-ozonated biofilters. The focus of this chapter is to identify seasonal trends in performance. Chapter 6 discusses nutrient availability in biofilters, through the use of the carbon:nitrogen:phosphorus (CNP) ratios. The references from all chapters are compiled in a single list at the end of the thesis. Several appendices are provided for additional detail.

Chapter 2
Background: Municipal Drinking Water Treatment Processes

The following sections present background information on the processes utilized at the Holmedale Water Treatment Plant (HWTP) in order of their position within the plant (starting with the raw untreated water). Although not included as part of the research project performed, brief background on ultraviolet light disinfection, chlorine disinfection, chloramination, and fluoridation are included for completeness.

2.1 Sand-ballasted Clarification

Sand-ballasted clarification (SBC), trade name ACTIFLO^{TM}, is a high rate clarification process which includes coagulation, flocculation and sedimentation. SBC functions through adding microsand which acts as a seed and ballast for floc formation (Desjardins *et al.*, 2002; Plum *et al.*, 1998). The addition of a ballasting agent, such as microsand, results in higher floc settling velocities because of increased floc density, larger floc size and greater roundness of flocs (Young & Edwards, 2003). Previous studies have also reported that ballasted flocs have velocity gradients more than ten times that of conventional flocculation (Imasuen *et al.*, 2004). The high settling rate and low breakup rate of ballasted floc translate into considerably shorter hydraulic retention time (HRT) in SBC units when compared to traditional coagulation-flocculation-sedimentation processes (Young & Edwards, 2003). The shorter HRTs enable SBC units to be considerably smaller than conventional clarification processes (Desjardins *et al.*, 2002). In drinking water applications, SBC has been shown to achieve turbidity removal rates of greater than 90%, produce water of equal quality to conventional coagulation-flocculation-sedimentation processes, and remove colour, algae and arsenic (Veolia Water Solutions & Technologies, 2007; Desjardins *et al.*, 2002; Plum *et al.*, 1998). In addition to its use for the treatment of surface water, SBC has also been used for numerous other applications such as the treatment of stormwater, combined sewer overflows, and wastewater (Plum *et al.*, 1998). SBC was first employed for drinking water treatment purposes in France, however its use has increased in North America, with 50 full-scale drinking water installations employed at the end of the year 2000 (Desjardins *et al.*, 2002; Plumb *et al.*, 1998).

The ACITFLO^{TM} process consists of three tanks in series, which function as injection, maturation, and lamella clarification tanks (Plum *et al.*, 1998). As the water flows into the injection tank the coagulant is added. A coagulant is added to either destabilize the colloids which are in stable

suspension in the water by charge neutralization or form a precipitate that will sweep the suspended particles down with it as it settles (Crittenden *et al.*, 2012). Following coagulant addition, water flows into the injection chamber, in which microsand and polymer are added under rapid mixing conditions (Plum *et al.*, 1998). The microsand and polymer are incorporated into the flocs, and act as ballasts. Water then flows into the maturation tank, in which the flocs are allowed to swell and mature (Plum *et al.*, 1998). The last step is the lamella clarification chamber, in which the large, heavy flocs settle and the clarified water leaves the process through weirs above the lamellas. Sludge and microsand from the bottom of the clarification chamber are recycled to the beginning of the process and are separated through a hydrocyclone (Plum *et al.*, 1998). The microsand is reinjected into the injection chamber, while the sludge is sent off to solids treatment (Desjardins *et al.*, 2002; Plumb *et al.*, 1998).

A limited number of peer-reviewed SBC studies have been published which include pilot- or full-scale removal data for these units, although numerous mentions of the use of SBC processes have been included in conference proceedings. Based on the available literature, SBC processes have been implemented with and without enhanced coagulation strategies, such as pH suppression, based on raw water characteristics and operational objectives (Cyna *et al.*, 2002). SBC units have also been used as pre-treatment strategies for nanofiltration membranes as well as biofilters (Cyna *et al.*, 2002). At a full-scale surface water treatment plant in France, SBC was shown to be an effective part of a multi-stage pre-treatment process for nanofiltration membranes (Cyna *et al.*, 2002). When investigating carbon removal through SBC, Rodriguez *et al.* (2007) reported total organic carbon (TOC) concentrations of 3.25 mg/L in SBC influent, and 2.04 mg/L in SBC effluent, which represented a 37% TOC removal through SBC. This was achieved at a full-scale water treatment plant treating highly coloured surface water, in which pre-chlorination, prior to SBC, was employed. SBC has also shown to be effective at removing other compounds such as phosphorus, nitrogen and heavy metals (Plum *et al.*, 1998). When used for polishing purposes, SBC removed 72% of total phosphorus from a river water source with an average total phosphorus concentration of 0.52 mg/L (n=5) (Plum *et al.*, 1998).

Published studies and industry experience demonstrate that SBC processes are effective when used in place of conventional coagulation-flocculation-sedimentation processes. As ballasting agents do not react chemically with flocs, it is speculated that these processes would not be substantially affected by cold water temperatures, as is the case with chemical and biological processes (Young & Edwards, 2003).

2.2 Ozone

Ozone is a common oxidant used in drinking water treatment for the oxidation of organics, such as eliminating colour, taste and odour-causing compounds, reducing natural organic matter (NOM), and destroying disinfection by-product (DBP) precursors (Crittenden *et al.*, 2012). In addition, ozone can also be applied for disinfection purposes (von Gunten, 2003). At the HWTP, ozone is applied prior to biofiltration with the main objective of removing taste and odour-causing compounds. These compounds can include geosmin, 2-methylisoborneol (MIB) and cyclocitral, which are produced naturally by cyanobacterial blooms (Crittenden *et al.*, 2012). The presence of taste and odour compounds in water, which cause a musty/earthy odour, is one of the main sources of customer complains to water utilities (Westerhoff *et al.*, 2006). The occurrence of taste and odor compounds is typically a seasonal problem, with a survey indicating that outbreaks in North American typically occur in the spring and summer (Suffet *et al.*, 1996). Ozone gas, which is typically generated onsite, for the use of taste and odour-causing compound removal is often added in doses of 1 to 3 mg/L with a minimum contact time of 10 to 15 minutes (Crittenden *et al.*, 2012). At the HWTP, an average yearly dose of 1 mg/L of ozone is added with the treatment objective being that there is zero ozone residual at the end of the ozone contact chambers.

Ozone is able to react with NOM, and taste and odour causing compounds in two ways; either directly with molecular ozone, or indirectly with hydroxyl radicals. Hydroxyl radicals are the strongest oxidants in water, and react fast with many dissolved compounds, while ozone is a much more selective oxidant (von Gunten, 2003). In natural waters, the presence of ozone initiators, promoters and scavengers determines to what extent ozone will be available as molecular ozone versus hydroxyl radicals, with NOM capable of acting as hydroxyl radical initiator, promoter and scavenger (Crittenden *et al.*, 2012; Langlais *et al.*, 1991). For MIB and geosmin, both ozone and hydroxyl radicals are powerful oxidants capable of their oxidation (Westerhoff *et al.*, 2006). Studies evaluating the oxidation of MIB and geosmin with ozone have shown that geosmin is more readily oxidized then MIB, and that in most cases molecular ozone is responsible for <20% of the removal of MIB and geosmin (Yuan *et al.*, 2013; Westerhoff *et al.*, 2006). This is made evident when comparing the rate constants of MIB and geosmin, with ozone and hydroxyl radicals. The rate constants of the odorants with hydroxyl radicals are approximately nine orders of magnitude greater than with molecular ozone (Westerhoff *et al.*, 2006). Therefore, reaction conditions leading to increased decomposition of ozone into hydroxyl radicals will lead to increased oxidation of MIB and geosmin.

Many factors, including pH, ozone dose, contact time, water quality, and temperature have been studied and can impact the ability of ozone to oxidize MIB and geosmin (Yuan *et al.*, 2013; Westerhoff *et al.*, 2006). Generally, these factors affect the oxidation of MIB and geosmin by impacting the availability of hydroxyl radicals. For example, the effect of pH on ozone can be explained due to the affinity of oxygen atoms with protons, which leads to increased decomposition of ozone in water at elevated pH. This leads to the availability of an increased number of hydroxyl radicals, which subsequently leads to greater removal of taste and odour compounds (Yuan *et al.*, 2013). Additionally, ozone decay is affected by temperature, with decreased decay at low temperatures (Gardoni *et al.*, 2012).

Although the use of ozone for the oxidation of MIB and geosmin is widely accepted, the production of potentially harmful ozonation by-products is of concern. Of greatest concern, is the ozonation by-product bromate, which is produced during ozonation of bromide-containing waters and has been classified by the United States Environmental Protection Agency (USEPA) as a probable human carcinogen (Crittenden *et al.*, 2012; von Gunten, 2003; USEPA, 1998b). To reduce bromate formation, many strategies have been employed including pH depression and ammonia addition. However, such strategies may lead to reduced oxidation of MIB and geosmin (Westerhoff *et al.*, 2006). Therefore, care should be taken when implementing bromate mitigation strategies to ensure sufficient MIB and geosmin removal are achieved.

In addition to its ability to remove taste and odour causing compounds, ozone also affects the character of NOM by creating low molecular weight biodegradable by-products. These low molecular weight compounds contribute considerably to the assimilable organic carbon (AOC) (Ramseier *et al.*, 2011; Hammes *et al.*, 2006; Huck, 1990), and biodegradable organic matter (BOM) (Rittmann *et al.*, 2002; Huck, 1990) fractions of water. If left untreated, these compounds can result in increased regrowth within distribution systems, increased chlorine demand, decreased biological stability, and affect corrosion of pipes (Hammes *et al.*, 2006; Escobar & Randall, 2001). Therefore, strategies, such as biofiltration, are often employed following ozonation to reduce the concentration of low molecular weight compounds (Urfer *et al.*, 1997; Huck *et al.*, 1991). Studies have reported that ozone is responsible for the formation of a large quantity of AOC (Ramseier *et al.*, 2011). Full-scale studies have identified an approximate 3-fold increase in AOC after pre-ozonation, with rapid sand filtration able to reduce the AOC back to pre-ozonated levels (Hammes *et al.*, 2006). The use of biofiltration, with sand, anthracite and granular activated carbon (GAC) media, after ozonation has resulted in

great AOC removal rates (Chien *et al.*, 2008; Hammes *et al.*, 2006). In their study, Chien *et al.* (2008) reported biofiltration AOC removal rates of 60% in GAC pilot columns, and 17% in anthracite pilot columns, when applied after ozonation. While Wert *et al.* (2008) observed up to 70% BOM (including AOC) removal through pre-ozonated pilot-scale anthracite biofilters.

2.3 Biofiltration

Biological filtration (biofiltration), in which bacteria attach to filter media and form a biofilm, has gained wide acceptance in North America as an effective process in drinking water treatment plants. The increased use of biofilters in water treatment is in part due to their ability to remove particles, similarly to traditional filters, and remove easily biodegradable compounds, including those produced during ozonation that can lead to regrowth within distribution systems (Urfer *et al.*, 1997). Other benefits of biofiltration include reduction in the formation of both carbon and nitrogen based disinfection by-products (DBPs), reduction in the chlorine demand, and control of taste and odour compounds, all of which can be achieved during biofiltration without the use of chemicals (Chu *et al.*, 2012; Urfer *et al.*, 1997). More recently, biofiltration has shown to be effective in removing trace contaminants, such as pharmaceuticals and endocrine disrupting compounds, and useful for membrane pre-treatment (Huck & Sozański, 2008). Many factors can impact the removal of BOM during biofiltration, including media, contact time, backwashing, temperature, influent BOM, and the quantity and activity of biomass present (Huck & Sozański, 2008).

One of the most important aspects of biofilter design is media selection, as it can considerably impact cost (Urfer *et al.*, 1997). Investigations have traditionally focused on adsorptive media, such as GAC, and non-adsorptive media, such as sand and anthracite (Urfer *et al.*, 1997). The increased porosity of GAC, compared to sand and anthracite, was historically thought to lead to greater biological activity within these biofilters, although studies have shown that this is not always the case. In part this is due to the inability of bacteria to fit within the micropores of GAC (AWWA, 1981). Wang *et al.* (1995) showed that the removal of TOC was no different between a pilot-scale wood-based GAC biofilter, an anthracite/sand biofilter and a sand biofilter, with TOC removals of 16%, 20% and 21% respectively. In the same study, the authors reported significantly higher TOC removal through GAC biofilters which had greater adsorption capacity, pointing to the importance of adsorption in removing TOC and reducing the trihalomethane (THM) formation potential in GAC biofilters (Wang *et al.*, 1995). Huck *et al.* (2000) also reported similar removal of biodegradable dissolved organic carbon (BDOC) through GAC/sand and anthracite/sand biofilters operated at

temperatures greater than 10°C. Therefore, studies suggest that once the adsorptive capacity of GAC has been exhausted, GAC and anthracite/sand biofilters perform comparably.

Empty bed contact time (EBCT), describes the time that water would spend in an empty filter and, when multiplied by the porosity, gives the time that the water is in contact with the biofilter media within a given contactor. EBCT is an important biofilter operating parameter as previous studies have demonstrated its effect on BOM removal (Urfer *et al.*, 1997). Hallé (2009) reported greater removal of TOC and dissolved organic carbon (DOC) through a pilot-scale anthracite/sand biofilter at 14 minute EBCT compared to a similar filter treating the same source water with 5 minute EBCT. The 14 minute EBCT filter achieved 19% and 16% removal, while the 5 minute EBCT filter achieved 13% and 11% removal, of TOC and DOC, respectively. Although EBCT has been shown to impact biofilter BOM removal, investigation into the effect of hydraulic loading on BOM removal has led Wang and Summers (1996) to state that substrate utilization, and not mass transfer, is the rate limiting step in BOM removal through biofilters. Numerous other studies have also demonstrated that hydraulic loading rate, within the typical range used for rapid filtration, does not impact BOM removal (Urfer *et al.*, 1997). Therefore, one strategy which can be used to increase BOM removal through biofilters is to increase EBCT by changing either the media depth or hydraulic loading rate of the biofilter (Urfer *et al.*, 1997).

Backwashing is another important operating parameter which can significantly impact biofilter operation. The objective of backwashing biofilters is similar to that of backwashing traditional filters; to remove entrapped particles, although attention must be paid to not severely disrupt the biomass (Urfer *et al.*, 1997). Numerous studies through the years have investigated backwashing processes to: determine optimal media bed fluidization to remove deposited materials, increase filter run times and effluent quality, and reduce mud ball formation (Slavik *et al.*, 2013). Studies have demonstrated that optimal backwashing procedures should include simultaneous water and air flow to achieve collapse-pulsing conditions (Amirtharajah, 1993) which can result in optimal filter cleaning, although backwashing procedure has not been found to have a considerable effect on biofilter BOM removal (Huck *et al.*, 2000).

The influent BOM concentration and composition to the biofilters determines the substrates available to the biomass for growth, and can vary considerably based on the source water used, and biofilter pre-treatment processes. Ozonation prior to biofiltration can substantially impact BOM concentration by increasing the biodegradability of NOM, as has been previously discussed (Volk &

LeChevallier, 2002). However, the use of oxidants upstream of the biofilters can considerably impact BOM removal, as residual ozone, chlorine and permanganate have been shown to inhibit biomass growth particularly in biofilters containing media other than GAC (Evans *et al.*, 2013a; Urfer *et al.*, 1997). In numerous studies, ozonation prior to biofiltration has been shown to increase the biomass quantity within biofilters, although increased BOM removal has not been reported with increased biomass quantity, quantified by the phospholipid method (Magic-Knezev and van der Kooij, 2004; Fonseca *et al.*, 2001).

Due to the impact of temperature on biological and chemical processes, it is expected that low temperatures will have an effect on biofilter performance. Such results have been reported by Emelko *et al.* (2006) who identified reduced oxalate removal at temperatures between 1 and 3°C compared to identical GAC and anthracite /sand biofilters operated between 21 and 25°C. However, in the same study the TOC removal of anthracite/sand biofilters remained between 15 and 20% at both temperatures between 1-3°C and 21-24°C (Emelko *et al.*, 2006). Moll *et al.* (1999) demonstrated significant reductions in BDOC removal through sand biofilters, with 38% removal reported in a sand biofilter operated at 5°C, compared to 60% BDOC removal reported for biofilters operated at 20 and 35°C.

As presented above, many factors influence BOM removal through biofilters, and as such, many of these factors also impact the quantity and activity of biomass present within biofilters. Numerous methods have been used to determine the quantity of biomass within biofilters, although the most widely used are the phospholipid and adenosine triphosphate (ATP) methods (Magic-Knezev & van der Kooij, 2004; Wang *et al.*, 1995). Due to the complex procedures involved in performing the phospholipid method, more recently, ATP based methods have gained in popularity. ATP is used for cell synthesis and maintenance as the main energy carrier in all living cells (Rittmann & McCarty, 2001). When cell death or injury occurs, ATP is released into the surrounding environments and rapidly utilized (Madigan & Martinko, 2006; Crouch *et al.*, 1993). Therefore, ATP provides a measure of the viable cells present within the biomass. The increased use of ATP analysis is especially interesting as studies have shown that the biomass quantity, as determined by the phospholipid method, is not correlated with the performance of biofilters in terms of BOM removal (e.g. Boon *et al.*, 2011). In addition to quantity determination, various methods have also been utilized to determine the activity of biomass within biofilters. Many of these methods involve

determination of the activity of certain key enzymes (Seredyńska-Sobecka *et al.*, 2006; Evans *et al.*, 2013a).

The many factors mentioned above should be considered when designing and operating biofilters, although in practice drinking water regulations or guidelines are set only for the biofilter effluent water turbidity. The "Guidelines for Canadian Drinking Water Quality: Supporting Documentation – Turbidity" indicates that the effluent of chemically assisted filtration treating surface water or groundwater under the influence of surface water should be below 0.1 NTU (Health Canada, 2003). The regulations or guidelines are based on turbidity, as the particles that contribute to turbidity may contain toxins, microorganisms and disrupt disinfection (Health Canada, 2003).

2.4 Ultraviolet Light Disinfection

In drinking water treatment plants, traditional disinfection utilizes oxidizing chemicals for disinfection, although more recently, the use of ultraviolet (UV) radiation has been applied. The benefit of UV disinfection is its ability to inactivate microorganisms, such as bacteria, viruses and protozoa, by transforming their deoxyribonucleic acid (DNA) which makes them unable to reproduce (Crittenden *et al.*, 2012; Dotson *et al.*, 2012). Additionally, as UV disinfection does not require the addition of chemicals, potentially harmful halogenated DBPs are not formed (Dotson *et al.*, 2012; USEPA, 2006). Although UV disinfection provides effective disinfection at the point of treatment, chemical disinfection is required to provide a residual through the distribution system in Canada (Health Canada, 2012).

UV light can be described as the electromagnetic radiation having a wavelength between 100 and 400 nm, just slightly shorter than the wavelength of visible light (Crittenden *et al.*, 2012). UV light with wavelength between 200 and 300 nm is known to have so called "germicidal" properties, because at these wavelengths the light is not absorbed by water, but it is absorbed by DNA (Crittenden *et al.*, 2012). At present, there are two types of UV lamps which are used commercially to produce UV light in the germicidal range, they include low-pressure (LP) and medium-pressure (MP) mercury vapor lamps (Dotson *et al.*, 2012). LP lamps emit UV light at a single wavelength of 253.7 nm and typically have lower energy outputs, while MP lamps emit UV light at wavelength from 200 nm to greater than 400 nm and can output significantly more energy (Dotson *et al.*, 2012). UV dose in drinking water treatment is expressed in mJ/cm^2 and determined based on the average UV intensity and the exposure time (Crittenden *et al.*, 2012). Factors such as the content of dissolved and

suspended substances in the water can impact UV dose by decreasing the UV intensity (Crittenden *et al.*, 2012). The average UV dose applied in drinking water treatment plants (DWTPs) surveyed in the United States using UV disinfection in conjunction with chlorine and chloramination was 40 mJ/cm^2, with one DWTP reporting an operating dose up to 180 mJ/cm^2 (Dotson *et al.*, 2012). At the HWTP, supplementary UV disinfection may be applied at certain times of year at a dose of 20 mJ/cm^2. However, during the present study, the UV disinfection remained on as part of the operating procedure for the first year of the upgraded plant. In the future, the use of quantitative microbial risks assessment may help guide the City of Brantford's operation of the UV process by identifying times of year when the supplementary UV disinfection is necessary. During the design of the new plant, recommendations were made suggesting that UV disinfection would be required when the pH of the source water was above 7.75 and temperatures were low, as during this time chlorine disinfection may not be able to achieve the design objectives for pathogen removal (R.V. Anderson Limited, 2007).

2.5 Chlorine Disinfection

Primary disinfection is used at water treatment plants to inactivate microorganisms, and the most common chemical disinfectant used in the United States is free chlorine (Crittenden *et al.*, 2012). Other chemical disinfectants used for primary disinfection include ozone and chlorine dioxide (Crittenden *et al.*, 2012). There are numerous advantages to using free chlorine, including its excellent effectiveness in disinfecting bacteria and viruses, although some disadvantages include the formation of regulated DBPs, and the poor disinfection of protozoa (Crittenden *et al.*, 2012). Although the use of chemical disinfectants in water treatment is widespread, the mechanisms by which microorganisms are inactivated are not well understood (Crittenden *et al.*, 2012). In DWTPs, disinfection is typically preceded by processes that remove particles and organic matter, to minimize the formation of DBPs and increase disinfection effectiveness (Health Canada, 2012).

Commonly, the product of the concentration of disinfectant (C, mg/L) and the contact time required to achieve a percentage of inactivation (t, minutes), known as Ct, is used to describe the dose of chemical disinfectant used (Crittenden *et al.*, 2012; Health Canada, 2012). The Ct required to inactivate a certain percentage of different microorganisms varies by up to six orders of magnitude depending on the disinfectant used, and is impacted by factors such as water temperature and pH (Health Canada, 2012; Jacangelo *et al.*, 2002). Greater Ct is required at high pH and at low temperatures, which in Brantford typically occur during winter months.

At the HWTP, the plant wide design objectives include: 2-log inactivation/removal of *Cryptosporidium*, 5.5-log inactivation/removal of *Giardia*, and 6.5-log inactivation/removal of viruses. These design objectives are based on USEPA best practices and *E. coli* data from the Grand River (R.V. Anderson Limited, 2007), and are calculated based on the free chlorine residual at the end of the chlorine contact chambers. As many of these design objectives cannot be achieved with chlorine disinfection alone at low temperatures, supplementary UV disinfection (as discussed in the previous section) is required. During chlorination, *Giardia* removal is used as the design objective, as chlorine is known to readily remove viruses, and is not effective against *Cryptosporidium* (R.V. Anderson Limited, 2007). Given the disinfection credits obtained from conventional filtration, 2-log removal *Cryptosporidium*, 2.5-log removal *Giardia*, and 2-log removal viruses (Ontario, 2006), chlorination must achieve at least 3-log inactivation of *Giardia*, to achieve the design objective of 5.5-log removal/inactivation. At the HWTP, the maximum flow rate through the plant which can be used to achieve 3-log removal *Giardia* with disinfection, assuming 2.5 mg/L free chlorine residual at the end of the chlorine contact chamber, varies between 38 MLD and 374 MLD (R.V. Anderson Limited, 2007). The great variation is due to the impact of temperature and pH on disinfection efficiency.

Although the primary objective of chemical disinfection is to inactivate microorganisms, the formation of DBPs associated with chemical disinfection must be considered. Optimal disinfectant doses should provide sufficient inactivation of microorganisms to ensure the safety of the drinking water, without causing considerable formation of DBPs. To help mitigate DBP formation during disinfection, processes ahead of disinfection should provide significant organic matter removal.

2.6 Chloramination

The final treatment step in many North American drinking water treatment plants is secondary disinfection, which ensures a disinfectant residual is maintained after treatment in the distribution system. Combined chlorine is typically used to provide a disinfectant residual. When chlorine is added to water which contains ammonia, chloramines, such as monochloramine, dichloramine and trichloramine, are formed (Crittenden *et al.*, 2013). These chloramines, in addition to the free chlorine, together are known as the total chlorine residual. If chlorine is added above a chlorine to ammonia molar ratio of one, any subsequent chlorine added reacts with the chloramines, and decreases the total chlorine residual. If chlorine addition is continued, the oxidation of chloramines continues until they are fully oxidized, which is called the “break point” (Crittenden *et al.*, 2012).

Following the breakpoint, any subsequent chlorine added contributes fully to the total chlorine residual (Crittenden *et al.*, 2012). Chloramines are used to provide a disinfectant residual because they are more effective than free chlorine in controlling microbial growth on pipe surfaces, and they are generally much more stable (LeChevallier *et al.*, 1988).

Over the course of 2012 at the HWTP, the average free chlorine residual at the end of the clear well, prior to ammonia addition, was 2.78 mg/L and the average ammonia dose applied was 1.26 mg/L.

2.7 Fluoridation

Fluoride is naturally occurring and can be found in soil, rocks and water (Jagtap, 2012). Fluorides are released into the environment by weathering processes, and find their way into the water supply by the dissolution of minerals in rocks and soil with which water is in contact (Jagtap, 2012; Health Canada, 2010). In some areas of the world groundwater has high fluoride concentrations due to its contact with rocks and soil, although some surface water sources have also been found to have elevated fluoride levels (Jagtap, 2012). Fluoride has been shown to protect tooth enamel from acids that may cause tooth decay, and subsequently leads to the prevention of dental cavities (Health Canada, 2010). The consumption of fluoridated drinking water has been shown in many studies to reduce the number of cavities in children (Health Canada, 2010). However, long term exposure to high levels of fluorides may lead to a condition called skeletal fluorosis, in which bones increase in density and become brittle (Health Canada, 2010).

In Canada, Health Canada has set a maximum allowable concentration of 1.5 mg/L of fluoride in drinking water, and recommends 0.7 mg/L fluoride in drinking water as the optimal concentration to promote dental health (Health Canada, 2010). The choice to add fluoride to drinking water is made by municipalities, in collaboration with the provincial and territorial authorities (Health Canada, 2010). The City of Brantford was the first Canadian municipality, which in 1945, implemented fluoride addition to the municipal water supply for the prevention of tooth decay (Rabb-Waytowich, 2009). At the time, the city was part of an 11 year case study, comparing the prevalence and severity of cavities with a neighboring city which did not practice fluoridation. Results of the study demonstrated the benefits of fluoridation for prevention and reducing the severity of cavities in children (Rabb-Waytowich, 2009). The raw water supply in Brantford has naturally occurring fluoride, and in 2012, the concentration was between 0.10 and 0.20 mg/L and no fluoride addition was undertaken (City of

Brantford, 2013b). Although fluoridation continues to be practised at the HWTP, it should be noted however that fluoride addition does not provide any treatment, rather the addition is done for public health reasons.

Although numerous governing bodies support the fluoridation of drinking water, such as the World Health Organization, Health Canada, and the Canadian Medical Association, numerous advocates oppose this practice. Arguments used against fluoridation include, the cost of fluoridation, environmental pollution, and health risks such as cancer, bone fractures, reproductive/developmental toxicity to name a few (Rabb-Waytowich, 2009). Such opposition has led to the discontinuation of fluoridation in numerous Canadian cities, with less than 50% of Canadian cities now practicing drinking water fluoridation (Rabb-Waytowich, 2009).

Of the treatment processes presented in the sections above, the research performed as part of this thesis focused specifically on SBC, ozonation, and biofiltration. Research was focused on these processes as they contribute to the largest fraction of NOM removal through the HWTP, and as limited data have previously been published relating to the full-scale performance of such processes. A discussion of the research gaps and needs addressed by this thesis can be found in Chapter 1, and in the introduction to each of the next four chapters, since this thesis is written in paper format.

Chapter 3
Assessment of Biomass in Drinking Water Biofilters by Adenosine Triphosphate

This chapter was submitted for potential publication in a scientific journal on December 30th, 2013. Therefore, it contains a separate overview, introduction, materials and methods, results and discussion, and conclusion. For additional background information, please see Chapter 2. References are compiled in the reference section at the end of this thesis.

3.1 Overview

Biofilters have gained in popularity for drinking water treatment to reduce disinfectant demand, disinfection by-product formation, and regrowth in distribution systems. Adenosine triphosphate (ATP) detection is being used more frequently as an easy and rapid method to quantify viable biomass in biofilters; however, there is little information on the value and relative performance of this method for biofilter applications. In this paper, a comprehensive comparison of published ATP data was conducted, and found that concentrations at the top of active, acclimated biofilters were typically in the range of 10^2 to 10^3 ng ATP/cm^3 media. The impact of various biofilter parameters (source water characteristics and quality including pre-treatment, hydraulic loading rate, temperature, sampling depth) on ATP levels is discussed and evaluated using published ATP data. The relationship between ATP and biofilter performance, in terms of carbon removal, is also assessed and indicates a need for further research in this area.

3.2 Introduction

Biological filtration (biofiltration) is gaining wider acceptance for drinking water treatment, and in 2013 the American Water Works Association hosted its first Biological Treatment Symposium. The increased use of biofilters in water treatment is in part due to their ability to remove easily biodegradable compounds, including those produced during ozonation, which can lead to regrowth in distribution systems (e.g. Urfer *et al.*, 1997). Other benefits include reduction in the formation of disinfection by-products (DBPs), reduction in the chlorine demand, and control of taste and odour compounds (Urfer *et al.*, 1997; Huck, 1990). The operation of biofilters is typically optimized for the removal of both particulate matter and biodegradable organic matter (BOM). To better understand the ability of biofilters to degrade BOM, many studies have included methods to measure the quantity

and activity of the biomass present within biofilters. Numerous methods have been developed or adapted for this purpose, including those that determine the concentration of adenosine triphosphate (ATP) present in the filters (e.g. Velten *et al.*, 2007). Although numerous models have been developed to predict biofilter performance (e.g. Rittmann & Stilwell, 2002), none have been found that include ATP as a measure of biomass quantity. ATP is the primary energy carrier in all living cells, and is used for cell synthesis and maintenance. Energy is released and made available to the cell through the hydrolysis of ATP, releasing phosphate and adenosine diphosphate (ADP). Only with the energy obtained from oxidation-reduction reactions in the cell can ADP gain phosphate and once again form ATP (Klingenberg, 2008). ATP is rapidly utilized by cells, and there is a rapid loss of ATP in dead cells following cell injury or substrate depletion (Crouch *et al.*, 1993). Therefore, quantification of ATP can provide a measure of viable biomass.

ATP-based methods have been used as an indicator of viable biomass in drinking water treatment biofilters in published studies (e.g. Velten *et al.*, 2011). However, there are little data available to provide guidance on levels of ATP that would normally be expected in active biofilters. Therefore, the present study included a survey and comparison of published data available on ATP in biofilters used for drinking water treatment. In addition, published studies were evaluated to determine if biofilter design and operating parameters can affect ATP concentration, and to determine if there is a relationship between biofilter performance and ATP concentration.

3.3 Methods to Measure Biomass Quantity and Activity in Biofilters

A number of analytical methods are available to measure the biological activity and quantity of microorganisms present in drinking water biofilters (Table 3.1). Biomass quantification in biofilters is often not done due to the complex analytical procedures involved and challenges in interpreting the results (Magic-Knezev & van der Kooij, 2004). In addition, there is a need for culture-free methods to assess the activity of microbial communities, since many types of microorganisms are non-culturable and result in an underestimation of the true value (Hammes *et al.*, 2010; Berney *et al.*, 2008). In selecting a method, care should be taken as certain methods measure the quantity of biomass while others measure the activity, and these may not be directly related (Table 3.1; Wang *et al.*, 1995). For biofilters, microbial activity will be important for BOM removal efficiency, and may be affected by various water quality parameters such as temperature, influent BOM concentration, and the presence of inhibitors such as chlorine (Wang *et al.*, 1995).

Table 3.1: Methods used to assess biomass in drinking water treatment biofilters

Measure	Method	Parameter measured	Advantages	Disadvantages	References
Biomass quantity	Total direct cell count (TDCC)	Microscopic enumeration	No incubation, good sensitivity	Time consuming	Velten *et al.*, 2007 Magic-Knezev & van der Kooij, 2004 Mauclaire *et al.*, 2004
	Heterotrophic plate count (HPC)	Growth on laboratory culture media	Simple, inexpensive	Many bacteria are non-culturable, requires long incubation	Evans *et al.*, 2013a Xiang *et al.*, 2013 Hammes *et al.*, 2010 Niemi *et al.*, 2009 Magic-Knezev & van der Kooij, 2004 Camper *et al.*, 1985
	Chloroform fumigation-extraction	Organic carbon released from microbial cells	Good sensitivity	Time consuming, complex, cannot differentiate live and dead cells	Campos *et al.*, 2002
	Phospholipid concentration	Phospholipids within cell membranes	Good sensitivity	Time consuming, complex, cannot differentiate live and dead cells	Xiang *et al.*, 2013 Emelko *et al.*, 2006 Seredynska-Sobecka *et al.*, 2006 Fonseca *et al.*, 2001 Huck *et al.*, 2000 Wang *et al.*, 1995 Findlay *et al.*, 1989
Biomass activity	Oxygen consumption	Aerobic respiration (Biomass respiration potential [BRP])	Rapid and simple once established	Difficult to establish method in biofilters	Xiang *et al.*, 2013 Urfer & Huck, 2001
	Tetrazolium salts (INT, CTC) reduction	Dehydrogenase activity	Rapid, simple, inexpensive	Poor sensitivity	Xiang *et al.*, 2013 Fonseca *et al.*, 2001
	Enzyme hydrolysis	Fluorescein diacetate [FDA] hydrolysis	Rapid, simple inexpensive	Does not measure all types of cells	Seredyńska-Sobecka *et al.*, 2006 Mauclaire *et al.*, 2004
	Enzyme hydrolysis	β-N-acetyl-hexosaminidase activity	Rapid, simple	Does not measure all types of cells	Evans *et al.*, 2013a

Measure	Method	Parameter measured	Advantages	Disadvantages	References
Quantity of active biomass	Adenosine triphosphate (ATP)	ATP within cells	Rapid, simple, sensitive	See discussion	See Table 3.3

Due to the challenges that exist with some biomass assessment methods listed in Table 3.1, the use of ATP analysis to quantify viable biomass in drinking water treatment biofilters has increased in recent years. ATP can be used to assess if the biomass of biofilters is stable or changing, and was recommended as a biological monitoring tool in a recent study whose aim was to assess practical monitoring and control methods for biological filtration (Evans *et al.*, 2013a). Advantages of using ATP-based methods are that they require little time and are simple to perform, limited laboratory equipment is needed, and the method is sensitive with low detection limits (Velten *et al.*, 2007). In addition, the ATP method is culture-free and can measure total viable cells including heterotrophic and autotrophic organisms.

ATP quantification is most often performed using a luminescent-based method, of which there are many commercial products on the market that have been developed to provide the reagents and instructions for ATP determination. Liquid chromatography-based methods for ATP determination have also been used, however, a disadvantage of this method is the high detection limit (2,800 ng ATP/g GAC) (Gibert *et al.*, 2013). Luminescent-based ATP methods consist of an initial physical, chemical or enzymatic cell lysis step which releases ATP from cells (Hammes *et al.*, 2010). A luciferase-luciferin complex is then added which reacts with the ATP to emit light, and the intensity is quantified using a luminometer (Hammes *et al.*, 2010; Magic-Knezev & van der Kooij, 2004).

The ATP method is typically used to measure the quantity of viable biomass attached to the surface of biofilter media, as it is this attached biomass that contributes to the measurable removal of BOM in biofilters (e.g. Urfer *et al.*, 1997). Previous studies that have applied the ATP method to biofilter media samples have used various methods for cell extraction and lysis. Some have used sonication of the biofilter media to detach the biomass from the biofilter media (Magic-Knezev & van der Kooij, 2004; Vahala *et al.*, 1998; Seger & Rothman, 1996), while others used a more rapid method consisting of direct lysis and quantification of ATP on the biofilter media without biomass detachment (Evans *et al.*, 2013a; Lauderdale *et al.*, 2012; Velten *et al.*, 2007). No studies have been

done to compare the different methods, and although ATP concentrations are measured using standards, it is possible that differences in cell extraction and lysis, sample handling, and processing times may cause a variation in results. In addition, it is also important to develop methods that minimize and evaluate potential contributions of free (extracellular) ATP when conducting analyses (Hammes *et al.*, 2010).

Determining ATP concentrations for a biofilter can be useful when looking at the effects of seasonal or operational changes on filter biomass. In many situations, data to monitor fluctuating ATP levels will provide sufficient information on the relative quantity of biomass in biofilters. In cases where an accurate microbial cell concentration is required, ATP per cell conversion ratios are required. ATP has been used to calculate the cell concentration in biofilters (e.g. Magic-Knezev & van der Kooij, 2004). However, the conversion of ATP to cell number has been identified as the largest problem with the interpretation of these results (Hammes *et al.*, 2010). The ATP content of cells can vary in different phases of growth and for different microbial species (Hammes *et al.*, 2010; Velten *et al.*, 2007). For this reason, case-specific ATP per cell conversion ratios should be developed for each process or environment of interest. Table 3.2 illustrates the variability of ATP per cell conversion ratios presented in the literature, with values that range from 10^{-10} to 10^{-5} ng ATP/cell, with the majority of data between 10^{-8} to 10^{-7}. This variation may be due to actual differences in ATP per cell ratios in microorganisms from different environments, but can also be affected by the method used to determine either the ATP or the cell concentration. In particular, results can depend on the method used to determine cell concentrations (Table 3.1). In situations where specific ATP per cell ratios have not been determined, it is more appropriate to present ATP concentrations instead of converting to cell numbers.

3.4 Factors Affecting ATP in Biofilters

Published studies that used ATP to measure the biomass in drinking water biofilters were surveyed, including both pilot- and full-scale biofilters (Table 3.3). Although the majority used fresh water as a source water, one study used sea water (Naidu *et al.*, 2013). To allow for a comparison of ATP values between studies, data in Table 3.3 were restricted to ATP concentrations from acclimated biofilters. In addition, Table 3.3 only includes data collected from the top 15 cm of the filter bed when available (the impact of sample depth on ATP is discussed later). ATP concentrations are presented in ng ATP/cm^3 media as a means of normalizing for different media types and densities, as has been done elsewhere in the literature (e.g. Urfer *et al.*, 1997). When the bulk density of the media was not

specified or could not be found from supplier sources, an average density for granular activated carbon (GAC) and sand of 0.5 and 1.5 g dry weight/cm^3, respectively, were used to convert from a mass basis (Urfer *et al.*, 1997; AWWA & ASCE, 1998). To standardize the reporting of ATP concentrations between studies, it is recommended that ATP be calculated and reported as ATP per unit media volume within the filter being investigated.

Table 3.2: Comparison of published ATP per bacterial cell ratios

ng ATP/cell*	Environment	References
2.1×10^{-8}	GAC filters	Magic-Knezev & van der Kooij, 2004
6.7×10^{-8} (σ 4.3×10^{-8})	Full-scale GAC filters	Velten *et al.*, 2007
8.9×10^{-8} (σ 1.07×10^{-7})	Aquatic environments (n=102; lakes, streams, groundwater, non-chlorinated drinking water, wastewater effluent, bottled water)	Hammes *et al.*, 2010
0.02 to 2.88×10^{-7}	Salt marsh creek	Wilson *et al.*, 1981
0.76 to 2.4×10^{-7}	Cell-bound ATP from planktonic bacteria in the different stages of a drinking water pilot plant	Hammes *et al.*, 2008
2.0×10^{-7}	Slow sand filters	Seger & Rothman, 1996
2.3×10^{-7} (σ 1.2×10^{-7})	Pilot-scale GAC filters	Velten *et al.*, 2007
3×10^{-7} (σ 1.5×10^{-7})	Pilot-scale GAC filters (n=105)	Velten *et al.*, 2011
3.6×10^{-7}	Rapid sand filters	Magic-Knezev & van der Kooij, 2004
1.5×10^{-10} to 5.5×10^{-7}	Groundwater	Metge *et al.*, 1993
2.2×10^{-7} to 3.6×10^{-5}	Groundwater	Eydal & Pedersen, 2007
0.2 to 7×10^{-7}	Water treatment membranes	Vrouwenvedler *et al.*, 1998
0.7 to 2.9×10^{-7}†	Starved subsurface bacterial isolates	Webster *et al.*, 1985

* Range or average and standard deviation (σ) values presented when available.
† Data published as ng ATP/CFU

In total, 16 published studies included ATP results from drinking water treatment biofilters (Table 3.3), the oldest of which was published in 1996. Seven studies presented full-scale biofilter ATP

concentrations, while the remainder were from pilot-scale biofilters. Pilot-scale biofilters have the advantage that they allow for easy media sampling, especially through the depth of the biofilter. Media sampling in full-scale biofilters is often more challenging, and depth sampling through the biofilter is not common practice. Of the studies reviewed, the typical ATP concentration in the top 15 cm of the filter bed was approximately 600 ng ATP/cm^3 media, and although there were substantial variations observed, concentrations were typically in the range of 10^2 to 10^3 ng ATP/cm^3 media. These benchmark ATP concentrations are beneficial in particular for water treatment plants that do not have historical data. However, Evans *et al.* (2013ab) recommends the collection of baseline biological parameters for each biofilter system, so that changes in ATP concentration by one or more orders of magnitude over time can be used to signal that the microbial community has undergone a significant change. The effect of biofilter design, operating parameters, and media sampling on ATP levels is discussed further below, and focuses on a select number of factors that are considered to most influence ATP concentration.

3.4.1 Temperature

Seasonal variations in water temperature can be substantial, and sometimes range by 20 to 30 C° (Moll *et al.*, 1999). Pharand *et al.* (2013) and Rahman (2013) found no relationship between the concentration of ATP and temperature in anthracite/sand biofilters over a temperature range of 3 to 28˚C, and 10 to 24˚C, respectively. Additionally, results from four full-scale water treatment plants, including anthracite, sand and GAC biofilters, revealed that ATP concentrations did not vary measurably over a nine month period during which there were substantial temperature fluctuations (Evans *et al.*, 2013a). Similar results have also been observed using the phospholipid method (Fonseca *et al.*, 2001). These results are different from what would be expected at cold temperatures, as bacterial growth rates and the kinetics of attachment are decreased at low temperatures (Huck *et al.*, 2000). For example, using the phospholipid method, Huck *et al.* (2000) reported a decrease in biomass at the top of both GAC and anthracite biofilters at temperatures between 1 and 3°C, compared to biofilters operated at temperatures between 21 and 25°C. Seger & Rothman (1996) have similarly shown that in slow sand filters ATP concentrations decreased at cold temperatures (less than 5°C).

Table 3.3: ATP concentrations measured at the top of acclimated drinking water treatment biofilters

Source water	Water temperature range (°C)	Biofilter media	Pre-treatment	Scale	Hydraulic loading rate (m/h)	EBCT (min)	ATP (ng ATP/cm^3)		ATP data points (n)	Influent DOC (mg/L)	DOC removal mean (%)	Reference
							Average	Range				
LAKES												
Lake Ontario, Canada	10 – 19	GAC	Ozone	Full	13.66	4-17	230	54-506	35	2.0	3	Wang & Siembida-Lösch, 2013
	3 – 14	GAC	None	Full	17.5	8-11	11	4-21	5	1.86	0	Siembida-Lösch, 2013
Lake Zurich, Switzerland[1]	7	GAC	Pre-filtration (20 µm), ozone	Pilot	5.9	15.76	585[2]	485-685[2] (σ)*	14	1.1	22	Velten *et al.*, 2011
Lake Zurich, Switzerland	NR	GAC	Ozone	Full	6.5	12.5	380	NR	NR	0.96	NR	Velten *et al.*, 2007
				Pilot	8	1.65	1,139	NR	NR	0.96		
Lake Arlington, USA[3]	11 – 30	GAC	Coag-floc-sed, ozone	Pilot	11	6[†]	NR	590-1,100	7	3.6	11	Lauderdale *et al.*, 2012
			Coag-floc-sed, ozone, nutrient addition		11		NR	600-1,500		3.6	20	
Lake Simcoe, Canada	NR	GAC	Ultrafiltration, UV	Full	NR	NR	NR	238-270[2‡]	NR	3.8-4.5[4]	NR	Taylor-Edmonds *et al.*, 2013
Lake P*ä*ij*ä*nne, Finland[5]	4 – 10	GAC	Coag-floc-sed, sand-filtration, ozone	Pilot	8	15	NR	685-1,342[5‡] (σ)*	2	2.6 (TOC)	12-14 (TOC)	Vahala *et al.*, 1998b
Lake M*ä*laren, Sweden	0 – 18	slow sand	Coag-floc, rapid sand filtration	Pilot	0.13	NR	NR	45-153[6‡]	NR	NR	5-20[7] (TOC)	Seger & Rothman, 1996
			Coag-floc, rapid sand filtration, ozone		0.13			68-158[6‡]		NR	5-30[8] (TOC)	

Source water	Water temperature range (°C)	Biofilter media	Pre-treatment	Scale	Hydraulic loading rate (m/h)	EBCT (min)	ATP (ng ATP/cm^3)		ATP data points (n)	Influent DOC (mg/L)	DOC removal mean (%)	Reference
							Average	Range				
RIVERS												
Grand River, Canada	3 – 28	A	Actiflo™, ozone	Full	3.19	38	1,268	705-2,037	28	3.98	12	Pharand *et al.*, 2013
Saugeen River, Canada	10 – 24	A	None	Pilot	5	10	163	73-294	22	4.21	5	Rahman, 2013
Grand River, Canada	1 – 23	A	None	Pilot	5	5	212	27-438	6	5.65	7	Hallé, 2009
					5	14	248	44-488	6	5.65	8	
Songhua River, China	22 – 24	GAC	None	Pilot	10.6[†]	10[†]	512[2,9]	NR	1	2.97-3.41 (TOC)	64 (TOC)	Zhang *et al.*, 2010
OTHER												
Bethune polder, Netherlands	3 – 24	GAC	Ozone	Pilot	3.7	35	150[10‡]	NR	NR	4.5-6	NR	van der Aa *et al.*, 2006
Netherlands (9 DWTP)	NR	GAC	Varied (± ozone)	Full	3-10	10-45	NR	24-5,067	30	1.8-5.4	NR	Magic-Knezev & van der Kooij, 2004
	NR	rapid sand			3-11	5-20	NR	16-2,592	9	2.0-3.2	NR	
	NR	slow sand			0.25-0.5	30-240	NR	18-93	3	1.4-3.2	NR	
United States (14 DWTP)	8 – 21	A, GAC & sand	Varied (± ozone)	Full	7.09	2.5-170	NR	1-70,000[11]	17	0.5-3.8	16	Evans *et al.*, 2013a
Sea Water, Chowder Bay, Australia	25	GAC	Centrifuge filtration	Pilot	10	3.9	13,500[12]	9,870-17,130 (σ)*	NR	1.85	57[13]	Naidu *et al.*, 2013
					7.5	5.4	13,200[12]	9,960-16,440 (σ)*		1.85	65[13]	
					5	7.8	15,300[12]	11,760-18,840 (σ)*		1.85	59[13]	

Table 3.3: ATP concentrations measured at the top of acclimated drinking water treatment biofilters (continued)

ATP values presented are from media samples collected from the top 15 cm of the filter bed, or as indicated. ATP values published on a per weight basis were converted to a volume basis (ng ATP/cm^3) using the bulk media density as specified.

A–anthracite, DOC–dissolved organic carbon, DWTP–drinking water treatment plant, EBCT–empty bed contact time, GAC–granular activated carbon, NR–not reported, S–sand, TOC–total organic carbon, UV–ultraviolet light.

* Upper and lower range of standard deviation (σ) included because maximum and minimum not available.

† Calculated using published filter surface area, filter bed volume and filtration rate.

‡ Media sample collection depth not specified.

[1] Residual ozone concentration is on average 0.22 mg/L and no backwashing was applied to the filter during this study (Velten *et al.*, 2011).

[2] Converted to volume basis using an average bulk density of GAC (0.5 g GAC/cm^3) (AWWA & ASCE, 1998).

[3] ATP concentrations extracted from Lauderdale *et al.* (2012) Figure 6 and presented in units of ng ATP/mL media.

[4] Influent DOC range from Taylor-Edmonds *et al.* (2013) Figure 2.

[5] Include both biofilters operated with and without nutrient addition, and converted to volume basis using average bulk density of Filtrasorb 400 GAC (0.54 g GAC/cm^3) (Calgon Carbon, 2012).

[6] ATP data from Seger & Rothman (1996) Figure 7, and converted to volume basis using an average bulk density of sand (1.5 g sand/cm^3) (Urfer *et al.*, 1997).

[7] Data from Seger & Rothman (1996) Figure 1.

[8] Data from Seger & Rothman (1996) Figure 2.

[9] Data from day 180 of filter acclimation study.

[10] Maximum average biomass concentration for the complete filter converted to volume basis using average bulk density of NORIT GAC 830 (0.5 g GAC/cm^3) (Norit Americas Inc., 2010).

[11] ATP concentrations extracted from Evans *et al.* (2013a) Figure 4.8 and presented in units of ng ATP/mL media.

[12] Converted to volume basis using bulk density of GAC provided by Naidu *et al.* (2013) (0.3 g GAC/cm^3).

[13] Average DOC removal in mature GAC calculated using data in Naidu *et al.* (2013) Table 3.

Although a number of studies have shown that the ATP level in biofilters remained essentially constant at varying temperatures, it is as yet unclear how this relates to biofilter performance in terms of dissolved organic carbon (DOC) and BOM removal. Research has shown that as temperatures decrease, DOC removal through biofiltration was reduced (Evans *et al.*, 2013a; Hallé, 2009; Fonseca *et al.*, 2001; Huck *et al.*, 2000; Moll *et al.*, 1999). This may be due to a reduced rate of enzymatic reactions at cold temperatures (Wolfenden *et al.*, 1999). Therefore, it is possible that the quantity of viable cells (as measured by ATP) could remain essentially constant at cold temperatures, but biodegradation of BOM could be reduced leading to decreased biofilter performance. This indicates that ATP might not be directly linked to biofilter performance, and that additional methods to monitor activity and/or measure the removal of specific compounds of interest are required.

3.4.2 Water source

The microbial and nutrient content of water used to feed biofilters can be expected to have an impact on ATP concentration. Although cell attachment to biofilter media depends on the properties of both the bacterial cell surface and the filter media, it has been suggested that high concentrations of microorganisms in water generally lead to high concentrations of attached biomass in biofilters (Wang *et al.*, 1995). Biomass detachment is also a key process in controlling biofilm growth, and can impact the quantity and activity of the biofilm (Stewart, 1993). The nutrient content of water, in terms of carbon, nitrogen and phosphorus concentrations, determines the level of substrate available for biomass growth (LeChevallier *et al.*, 1991). In drinking water treatment plants, organic carbon is often the growth-limiting nutrient (LeChevallier *et al.*, 1991) although there are some examples where phosphorus is the limiting nutrient (Lehtola *et al.*, 2001). Carbon availability is often monitored using total organic carbon (TOC) and DOC. However, these methods do not measure the biodegradable dissolved organic carbon (BDOC) fraction (Volk & LeChevallier, 2000).

As the concentration and biodegradability of BOM available to the biomass can be impacted by pre-treatment processes, such as ozonation (discussed in the subsequent section) it is reasonable to think that the concentration and composition of BOM in the biofilter influent would have a greater impact on ATP concentration than the water source. This is confirmed by evaluating the results in Table 3.3, which show no substantial difference in ATP concentration in media at the surface of biofilters fed with river or lake water. It may be that the BOM was similar in biofilters fed from rivers and lakes, either at the source or following pre-treatment steps.

Further analysis of the data from Table 3.3 shows an increasing trend in ATP concentration with increasing influent DOC for biofilters without ozone pre-treatment, although only limited data are

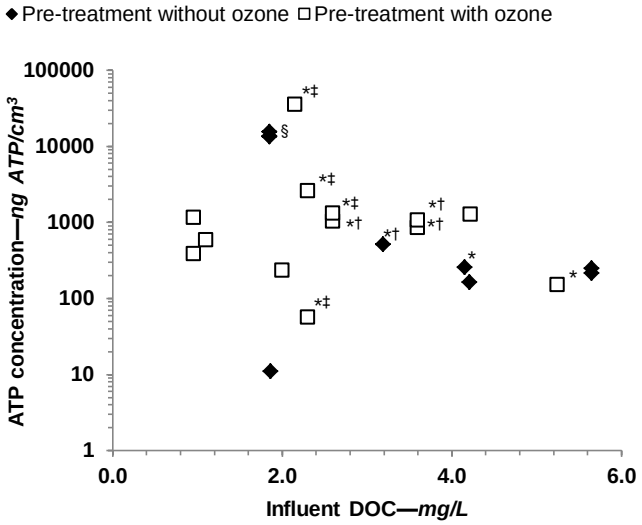
available (Figure 3.1). An exception is the ATP concentration in GAC media from sea water-fed biofilters (Naidu *et al.*, 2013), which had substantially higher ATP concentrations compared with the other studies. There was no relationship between influent DOC and ATP concentration of biofilters with ozone pre-treatment (Figure 3.1). Which suggests that DOC composition, and in particular the biodegradable fraction, is important for microbial growth and activity. In future studies, emphasis should be placed on determining the concentration and composition of BOM in the biofilter feed water to investigate the possible relationship between BOM and ATP.

3.4.3 Pre-treatment

Ozone is often employed in drinking water treatment plants for pathogen inactivation and also to reduce taste and odour compounds, and disinfection by-product precursors (Camel & Bermond, 1998). However, ozone also increases the concentration of BOM in water (e.g. Huck, 1990). Elevated BOM levels can lead to increased bacterial regrowth in distribution systems and increased chlorine demand (e.g. Huck *et al.*, 2000). For this reason, utilities often use biofiltration after ozonation to reduce the BOM concentration. When employing ozone prior to biofiltration, care must be taken to ensure no ozone is carried onto the biofilters, as concentrations as low as 0.1 to 0.2 mg/L of residual ozone can inhibit bacterial development in biofilters (Urfer *et al.*, 1997). Carryover of other oxidants, such as chlorine and permanganate, should also be avoided as their presence in the biofilter influent has been shown to decrease the ATP concentration at the surface of biofilters (Evans *et al.*, 2013a).

In pre-ozonated biofilters, the concentration of carbon available for microbial growth is greater than that of non-ozonated biofilters, leading to increased biological activity (Urfer *et al.*, 1997). Magic-Knezev and van der Kooij (2004) found that pre-ozonation of water used for full-scale GAC biofilters increased the ATP concentration in the top layer of the filter bed by 2 or 3 times compared to biofilters under similar operating conditions fed with non-ozonated water. Similar results were also observed in full-scale biologically active GAC filters at the Lakeview Water Treatment Plant in Ontario, Canada. In that study, when pre-ozonation was applied, a considerable increase in ATP concentration at the surface of biofilters was observed (Wang & Siembida-Lösch, 2013; Siembida-Lösch, 2013). In another study, using an INT method to measure biomass, results from pilot-scale sand filters showed 55% higher dehydrogenase activity in ozonated water compared to identical biofilters operated without ozone (Fonseca *et al.*, 2001). The studies in Table 3.3 show that the addition of ozone prior to GAC biofilters, operated at various temperatures, lead to ATP concentrations two to three times higher than biofilters operated without ozone pre-treatment. Due to the limited data available, the effect of ozone pre-

treatment on the concentration of ATP in anthracite and sand filters, and in biofilters treating sea water could not be determined.



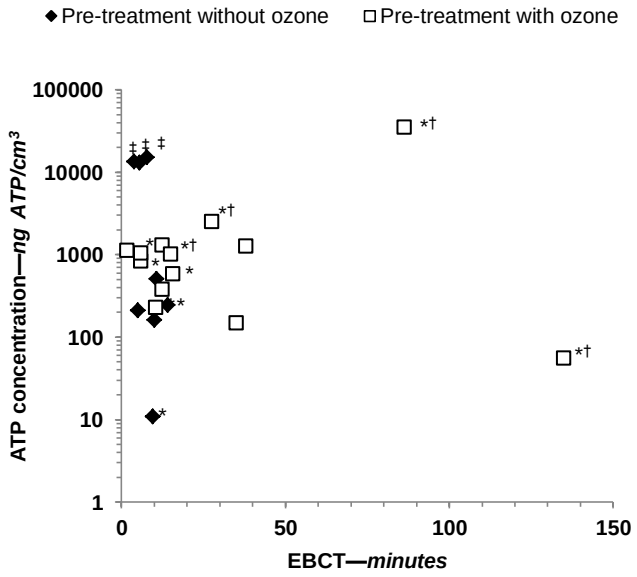
* When mean ATP concentration or influent DOC data were not available, the median of the range was used.
† TOC removal used instead of DOC removal.
‡ Includes both pre-treatment with and without ozone as data separated based on pre-treatment were not available. The inclusion of these data points does not appear to affect the relationship observed within the figure.
§ Naidu *et al.*, 2013 (sea water)
Studies not included from Table 3.3 are those that did not report influent TOC/DOC.

Figure 3.1: Mean ATP concentration at the surface of biofilters vs. influent dissolved organic carbon (DOC) to the biofilters

3.4.4 Hydraulic loading and contact time

Hydraulic loading rate and empty bed contact time (EBCT) are a function of the volumetric flow rate of water through biofilters. EBCT is a measure of how long water is in contact with the biofilter media within a given contactor and is a key biofilter operating parameter. Hydraulic loading rate measures the flux (rate of mass flow per unit area) of water onto biofilters. Thus for a given filter, an increase/decrease in hydraulic loading rate leads to a decrease/increase respectively in EBCT. However, when comparing filters from different studies, there is no relationship *per se* between nutrient flux and EBCT. Both Hallé (2009) and Naidu *et al.* (2013) have shown that EBCT did not affect ATP concentrations in pilot-scale biofilters. A previous study has shown that at a given EBCT, BOM removal through biofilters, quantified by DOC, assimilable organic carbon (AOC) and BDOC, is not impacted by hydraulic loading rate in the range of 1.5 to 15 m/h (Wang & Summers, 1996). Since

hydraulic loading rate governs organic matter flux, it may affect ATP concentrations. Further analysis of data in Table 3.3 shows that there is no relationship between EBCT and ATP at the surface of biofilters (Figure 3.2). Instead, as would be expected, results show a general trend of increasing ATP concentration with increasing hydraulic loading rate (Figure 3.3). The scatter in the data are no doubt related to the other confounding factors that can influence ATP concentration (e.g. backwash frequency and efficiency). More comparative studies to assess the effects of EBCT and hydraulic loading on ATP are required.



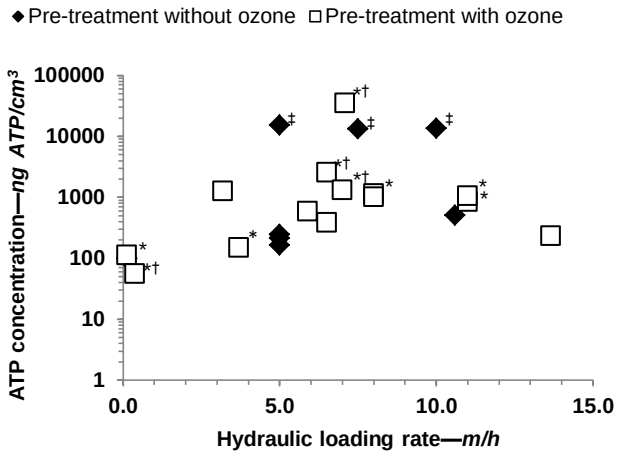
EBCT—empty bed contact time.
* When mean ATP concentration or EBCT data was not available, the median of the range was used.
† Includes both pre-treatment with and without ozone as data separated based on pre-treatment were not available. The inclusion of these data points does not appear to affect the relationship observed within the figure.
‡ Naidu *et al.*, 2013 (sea water)
Studies not included from Table 3.3 are those that did not report EBCT.

Figure 3.2 Mean ATP concentration at the surface of biofilters vs. biofilter EBCT

3.4.5 Media type

Media selection is one of the central factors in biofilter design primarily because it has a great impact on cost (Urfer *et al.*, 1997). Investigations into adsorptive media, such as GAC, and non-adsorptive media, such as anthracite and sand, have been the primary focus of biofiltration studies (e.g. Huck *et al.*, 2000; Urfer *et al.*, 1997). Emelko *et al.* (2006) found that at temperatures between 21 and 25˚C, biomass quantity measured using the phospholipid method (nmol P/cm^3 media), was higher in the

surface layer of anthracite/sand biofilters compared to GAC biofilters, of which the adsorption capacity was essentially exhausted. However, at temperatures between 1 and 3 ˚C, there was no difference in biomass concentration between biofilters of different media type (Emelko *et al.*, 2006). Evans *et al.* (2013a) demonstrated that the ATP concentration (reported as ng ATP/mL of filter media) in biofilters with GAC media was not consistently higher than in anthracite or sand media. On the contrary, results from Wang *et al.* (1995) using the phospholipid method showed that the attached biomass in the top of biofilters containing GAC or sand were similar, and both were higher than anthracite media (when converted to a nmol lipid-P/cm^3 basis as described by Urfer *et al.*, 1997). Similar trends of increased biomass attached to GAC media compared to anthracite, expressed as nmol P/cm^3 media, have also been reported by Huck *et al.* (2000) in filters at a location in California. Although virtually no biomass growth has been observed in the micropores of GAC due to the inability of bacteria to fit within their small diameter (AWWA, 1981), the increased irregularity and macropores of GAC may protect the biomass from shear stresses, which is thought to lead to a greater degree of biomass attachment (Urfer *et al.*, 1997).



* When mean ATP concentration was not available, the median of the range was used.
† Includes both pre-treatment with and without ozone as data separated based on pre-treatment were not available. The inclusion of these data points does not appear to affect the relationship observed within the figure.
‡ Naidu *et al.*, 2013 (sea water)
Studies not included from Table 3.3 are those that did not report influent hydraulic loading rate.

Figure 3.3: Mean ATP concentration at the surface of biofilters vs. hydraulic loading rate

The ATP concentrations in the surface layer of anthracite and GAC biofilters in Table 3.3 are generally in the same order of magnitude (10^2 to 10^3 ng ATP/cm^3 media), although only limited data are

available for anthracite biofilters. Two studies (Magic-Knezev & van der Kooij, 2004; Seger & Rothman, 1996) that provide ATP data in media collected from the top of sand filters had results that were generally one order of magnitude lower than anthracite or GAC biofilters. However, these slow sand biofilters had very low hydraulic loading rates and would be expected to have low concentrations of biodegradable organics in the influent water due to their location within the treatment plants (preceded by various treatment steps). Therefore, further studies designed to compare the concentration of ATP in biofilters with different media types are recommended.

3.4.6 Biofilter sampling

The location and timing of media collection from biofilters may affect ATP concentrations. Studies on the effect of sample depth have been performed using ATP to evaluate biomass through biofilters (Table 3.4). Zhang *et al.* (2010) found that there was a decrease in ATP concentration with depth in a GAC biofilter, but that these differences decreased as the biofilter became acclimated. The ATP concentrations at the end of the study (day 180) were 512, 497 and 468 ng ATP/cm^3 media in the top, middle and bottom of the filter, respectively. Rahman (2013) also observed decreases in ATP concentration through filter bed depth in a pilot-scale anthracite/sand biofilter. van der Aa *et al.* (2006) found that the ATP concentration was 50% higher at the top of a GAC biofilter when compared to the middle of the biofilter. Investigations using phospholipid analysis have also found that biomass decreased through biofilter bed depth (Xiang *et al.*, 2013; Hallé, 2009; Emelko *et al.*, 2006; Persson *et al.*, 2006; Urfer & Huck, 2001; Huck *et al.*, 2000; Wang *et al.*, 1995).

Other studies have shown either no change or an increase in biomass over filter depth. Evans *et al.* (2013a) reported similar ATP concentrations at the top and bottom of four full-scale biofilters of different media types. Hallé (2009) observed increasing ATP concentrations through the entire bed depth in pilot-scale anthracite/sand filters, with elevated ATP in the middle of the biofilter at low temperatures. Velten *et al.* (2011) also reported an initial increase in ATP concentration with bed depth, followed by a subsequent decrease by a factor of 2.3 to the bottom of a non-backwashed pilot-scale GAC biofilter. However, others have explained that low concentrations of biomass at the surface of biofilters may be caused by the presence of inhibitors such as residual ozone in the influent of biofilters (Evans *et al.*, 2013a; Urfer *et al.*, 1997).

Table 3.4 ATP concentration through biofilter bed depth

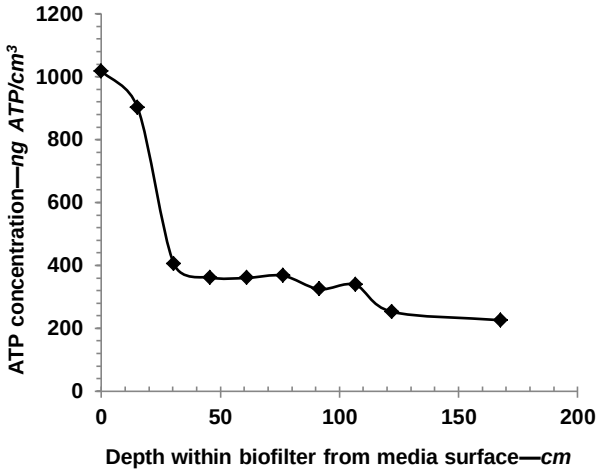
Source Water	Pre-treatment	EBCT* (min)	Total biofilter depth (cm)	ATP (ng ATP/cm^3)		Sample location	Media sampled	Reference
				Average	Range			
Saugeen River, Canada[1]	None	10	80	221 180 141	142-298 130-259 104-180	Top Middle Bottom	Anthracite Anthracite Sand	Rahman, 2013
Lake Zurich, Switzerland[2,3]	Pre-filtration (20µM), ozone	15.76	155	585 915 590 400	485-685 (σ)† 715-1115 (σ)† 490-690 (σ)† 300-500 (σ)†	Top Middle Middle Bottom	GAC GAC GAC GAC	Velten *et al.*, 2011
Songhua River, China[3,4]	None	10	NR	NR NR NR	397-512 255-497 200-467	Top Middle Bottom	GAC GAC GAC	Zhang *et al.*, 2010
Grand River, Canada[5]	None	14	117	248 430 593	44-488 46-690 266-847	Top Middle Bottom	Anthracite Anth./sand Sand	Hallé, 2009

Anth./sand–anthracite/sand interface, EBCT–empty bed contact time, NR–not reported.
*Total EBCT of biofilter.
† Upper and lower range of standard deviation (σ) included because maximum and minimum not available.
[1] Samples collected at 8 cm, 28 cm, and 67 cm below anthracite media surface in dual media (anthracite/sand) filter.
[2] Samples collected at 10 cm, 45 cm, 80 cm, and 115 cm below media surface.
[3] Concentration of ATP converted to volume basis using an average bulk density of GAC (0.5 g GAC/cm^3) (AWWA & ASCE, 1998).
[4] Samples collected at 140 cm, 100 cm, and 60 cm above filter bed base. ATP from day 60 to day 180 extracted from Figure 3 in Zhang *et al.*, 2010.
[5] Samples collected at 5 cm, 15 cm, and 59 cm below media surface.

Although most studies indicate that the concentration of ATP decreases through biofilter bed depth, further studies should be performed to gain a better understanding of this relationship. To address this knowledge gap, the authors performed ATP measurements on media samples from a full-scale anthracite/sand biofilter at the Holmedale Water Treatment Plant in Brantford, Ontario, Canada. The plant is fed using river water, and further details are provided in Pharand *et al.* (2013) and in Table 3.3. Core samples of media were collected immediately following draining of a filter after 60 hours of operation but prior to backwash. Approximately 30 g of media were collected at each sample depth, mixed, and 1 g of each sample was measured using the LuminUltra Deposit & Surface Analysis (LuminUltra Technologies Ltd., Fredericton, New Brunswick, Canada) test kit. Results showed that ATP concentrations were highest at the top of the biofilter, with values of 1018 and 901 ng ATP/cm^3 media at 0 and 15 cm depths, respectively (Figure 3.4). Within the anthracite layer, ATP levels steadily

decreased to approximately 253 ng ATP/cm^3 media at a depth of 122 cm, and decreased further from the anthracite into the sand. As was expected, these results suggest that stratification of ATP through the depth of a full-scale biofilter follows a similar trend to those observed in pilot-scale filters (Rahman, 2013; Velten *et al.*, 2011; Zhang *et al.*, 2010).

The time that samples are taken in relation to filter backwash, and also backwash conditions, could also affect ATP concentrations, however there is no information on this in the literature. Data from Table 3.3 could not be assessed for the effect of backwash conditions, including the time of media collection relative to backwash, as most studies did not include this information. A study that used phospholipids to measure biomass showed that backwash conditions had essentially no effect on biomass quantity at the surface of full-scale biofilters or on BOM removal (Huck *et al.*, 2000). However, more research is needed to determine if filter run time and backwash can affect ATP concentration, and to provide recommendations on the optimal timing and location for sample collection for biomass measurements. In addition, further studies should be done to compare the variation in ATP concentration in full-scale and pilot-scale biofilters, even though it would be expected that the stratification of media and backwash effects in an appropriately designed and operated pilot-scale biofilter would mirror that of a full-scale filter.



Samples analyzed above 150 cm were anthracite, and at 160 cm was from the sand layer.

Figure 3.4 ATP concentration through the depth of a full-scale pre-ozonated, dual media (anthracite/sand) biofilter

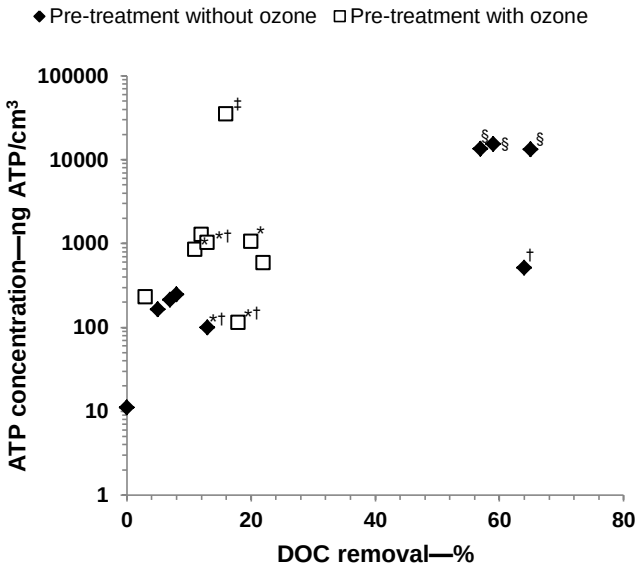
3.5 Relationship Between ATP and Biofilter Performance

Typical biofilter performance measures include TOC, DOC and AOC removal. In this paper, while recognizing its limitations, DOC was used as the main biofilter performance indicator due its common use in water treatment studies, and its ability to describe overall filter performance with respect to organics removal (Table 3.3; Velten *et al.*, 2011). Other performance indicators, such as removal of AOC and BDOC, were not considered due to the limited number of studies published with both ATP and AOC/BDOC data. In addition, previously published results show no correlation between AOC concentration and biomass quantity, measured using an ATP method, within GAC biofilters (Velten *et al.*, 2011). In the six studies reviewed in this paper that evaluated AOC (Evans *et al.*, 2013a; Naidu *et al.*, 2013; Pharand *et al.*, 2013; Siembida-Lösch, 2013; Wang & Siembida-Lösch, 2013; Vahala *et al.*, 1998), removal through biofilters was between 35 and 90% with no apparent relationship between AOC removal and ATP at the surface of biofilters. However, Naidu *et al.* (2013) reported the highest AOC and DOC removal rate (90 and 59%, respectively), and also had the highest biofilter ATP concentrations. Although AOC and BDOC do not encompass all BOM available for microbial growth, they are useful measures of the fraction of organic carbon that can be assimilated biologically (Volk & LeChevallier, 2000). $BDOC_{filter}$, which measures DOC reduction using a 60 minute shaker-type BDOC test, may also be a useful indicator of DOC removal through biofiltration (Carlson & Amy, 1997).

Studies in Table 3.3 that assessed both ATP and mean DOC removal values were compared to investigate if there is a relationship between the two parameters (Figure 3.5). The results were skewed by the sea water study from Naidu *et al.* (2013) that showed the highest ATP and DOC removal values. By excluding this study, there was essentially no relationship between DOC removal and ATP concentration (Figure 3.5). Results from full-scale (Pharand *et al.*, 2013) and pilot-scale (Hallé *et al.*, 2009) biofilters also showed that ATP concentration was not related to DOC removal. Both of these studies used feed water from the same river (Grand River, intakes approximately 55 km apart) and had similar DOC removal rates, but the ATP concentration at the full-scale plant was approximately 6 times higher than the pilot-scale biofilter. It is likely that pre-treatment (full-scale with pre-treatment including ozone versus pilot-scale without pre-treatment) influenced these results.

Only a limited number of studies have shown a relationship between organic carbon removal and ATP concentration in biofilters. Lauderdale *et al.* (2012) reported that a GAC biofilter with nutrient dosing (orthophosphate) had a higher maximum concentration of ATP at its surface (1,500 ng ATP/mL), and achieved on average 9% higher DOC removal compared to a reference GAC biofilter

without nutrient addition. Seger and Rothman (1996) also found that an increase in ATP at the surface of slow sand filters corresponded with an increase in TOC removal, and that this trend was apparent in filters with and with-out pre-ozonation. Using the phospholipid method, Wang *et al.* (1995) found that an anthracite biofilter with less attached biomass than carbon-based GAC biofilters also had significantly reduced biofilter performance in terms of TOC and DBP precursor removal. However, in the same study, a wood-based GAC biofilter with a higher concentration of biomass than an anthracite biofilter performed equally in terms of TOC and DBP precursor removal.



DOC—dissolved organic carbon.
* When mean ATP concentration or DOC removal data were not available, the median of the range was used.
† TOC removal used instead of DOC removal.
‡ Includes both pre-treatment with and without ozone as data separated based on pre-treatment was not available. The inclusion of these data points does not appear to affect the relationship observed within the figure.
§ Naidu *et al*., 2013 (sea water)
Studies not included from Table 3.3 are those that did not report TOC/DOC removal.

Figure 3.5 Mean ATP concentration at the surface of biofilters vs. mean DOC removal through biofilters

The possible relationship between ATP concentration and biofilter performance is especially interesting as previous publications have shown that the performance of biofilters in terms of BOM removal was not directly correlated with biomass quantity as measured by the phospholipid method (e.g. Boon *et al.*, 2011; Huck & Sozanski, 2008). As only limited results are available comparing organic carbon removal with ATP, it is recommended that additional research be performed. Also, future studies should

incorporate methods that specifically measure BOM as opposed to total carbon. The results will ultimately help determine if ATP analyses can provide a good measure of viable biomass present within biofilters, and if that amount can be related to overall biofilter performance.

3.6 Conclusion

ATP can be used to measure the amount of viable biomass within drinking water treatment biofilters, and newer ATP-methods are simple and less time consuming than other biomass quantification methods. Due to these advantages, ATP-based methods are gaining popularity in the assessment of biofilter biomass. Based on a review of published studies available to-date that have used ATP to measure the biomass of drinking water biofilters (ie. ATP per unit filter volume), the following can be concluded:

- Water source, temperature, EBCT, and the use of anthracite versus GAC media do not impact the ATP concentration at the surface of acclimated biofilters.
- In some studies, increasing influent DOC and hydraulic loading rate increased ATP concentration at the surface of acclimated biofilters.
- An ATP concentration of 10^2 to 10^3 ng ATP/cm^3 media appears to represent active, acclimated biofilters for both GAC and anthracite biofilters. For biofilters with ATP concentrations less than this benchmark, further investigation is recommended.
- Limited data are available on ATP concentrations at the surface of anthracite, and sand filters, therefore additional research should be undertaken to further evaluate biofilters that contain these media types.
- Pre-treatment of biofilter feed water with ozone typically results in a two to three fold increase in ATP concentration at the surface of biofilters.
- ATP levels at the surface of the biofilter are not necessarily related to biofilter performance, in terms of DOC removal.
- The majority of published studies have shown that ATP concentrations decrease through the depth of biofilters, although not all studies have supported this finding. Further investigation into the effect of sample depth on ATP concentration should be undertaken, taking into consideration media type (anthracite, GAC, and sand), temperature, and filter backwashing.

3.7 Disclaimer

Mention of trade names or commercial products does not constitute endorsement or recommendation for their use by the authors or funding agencies.

Chapter 4
Natural Organic Matter Removal by Sand-ballasted Clarification for Drinking Water Treatment

This chapter was submitted for potential publication in a scientific journal on April 7, 2014, and as such includes an overview, introduction, materials and methods, results and discussion, and conclusion. References were compiled in the reference section at the end of this thesis.

4.1 Overview

Natural organic matter (NOM) is a complex group of compounds which can impact the selection and operation of drinking water treatment processes. Coagulation is typically one of the first processes in drinking water treatment used to remove NOM. This study assessed NOM removal by coagulation at a full-scale municipal drinking water treatment plant, and both new and traditional analytical techniques were used to measure specific NOM fractions. The sand-ballasted clarification (SBC) process used at the plant combines coagulation, flocculation, and sedimentation, and achieved 30% total organic carbon (TOC) removal under conditions that included average raw water TOC of 6.31 mg/L, alkalinity of 196 mg CaCO3/L, and post-sedimentation pH of 7.6. The use of an advanced NOM characterisation technique, liquid chromatography-organic carbon detection (LC-OCD) demonstrated 53% biopolymer and 41% humic substance removal through the SBC process. The removal of biopolymers is important in water treatment due to their role in low pressure membrane fouling, and the removal of humic substances is important as they contribute to disinfection by-product formation. Traditional humic substance surrogate analyses were also performed, including ultraviolet absorbance at 254 nm (UVA_{254}) and there were similarities between the removal of LC-OCD-quantified humic substances and UVA_{254} reduction through SBC.

4.2 Introduction

Natural organic matter (NOM) in water describes the matrix of organic compounds which originate from living organisms, and includes both high and low molecular weight compounds such as carboxylic acids, carbohydrates, proteins, humic and fulvic acids (humic substances) and lipids (Matilainen *et al*., 2000). NOM composition can vary considerably in water due to local environmental conditions, although humic substances typically constitute the largest fraction (Thurman, 1985). The removal of NOM through drinking water treatment plants (DWTPs) is important as it can affect the aesthetic

quality of water due to compounds that contribute to increased colour, taste, and odour. In addition, some NOM fractions can increase disinfectant and coagulant demand, reduce the adsorptive capacity of activated carbon, and contribute to corrosion and microbial regrowth in distribution systems (Jacangelo *et al.*, 1995). Biopolymers (proteins and polysaccharides) have also been shown to contribute to hydraulically reversible fouling of low pressure membranes (Hallé *et al.*, 2009; Peldszus *et al.*, 2011; Tian *et al.*, 2013). Humic substances are of particular importance as they have been shown to act as precursors to potentially harmful disinfection by-products (DBPs) (Singer, 1999). In Canada, maximum acceptable concentrations of organic DBPs in finished water have been set for total haloacetic acids (HAAs), N-nitrosodimethylamine (NDMA), and trihalomethanes (THMs) (Health Canada, 2012), and in the United States maximum concentration levels (MCLs) are established for THMs and HAAs. The World Health Organization (WHO) has also set guideline values for numerous DBPs, which include THMs, and NDMA while also recognizing many additional DBPs which occur in DWTPs for which guideline values are not yet established (WHO, 2011). Many other jurisdictions around the world, including those in the European Union and Australia, have also established guidelines for acceptable concentrations of DBPs in finished water (e.g. NHMRC and NRMMC, 2011).

In water treatment systems, coagulation has traditionally been used to decrease colour and remove particles and pathogens, although it is also employed for the removal of dissolved NOM (Edzwald, 1993; Matilainen *et al.*, 2010). Due to its complex nature, dissolved organic matter is often expressed in terms of dissolved organic carbon (DOC), which typically makes up a large component of total organic carbon (TOC). Coagulation can lead to particle and TOC removal by either destabilizing colloids and reducing their surface charge to favour aggregation during flocculation (i.e. charge neutralization) or by the adsorption of compounds onto the precipitate of metal coagulants (i.e. sweep flocculation) (Cheng *et al.*, 1995). Enhanced coagulation strategies can be used to increase TOC removal and most often include pH suppression, although changing coagulant type or dose and using a coagulant aid, along with pH suppression can also be used (and to ensure that changes being made to coagulant dose do not compromise turbidity removal) (Yin *et al.*, 2006; MOE, 2010). A modified coagulation process is sand-ballasted clarification (SBC), which includes up-flow sand-ballasted coagulation, flocculation, and sedimentation in one unit process. SBC employs microsand which acts as a seed and ballast for floc formation (Desjardins *et al.*, 2002; Plum *et al.*, 1998), and results in increased floc settling velocity which leads to shorter hydraulic retention time (HRT) (Young & Edwards, 2003). In drinking water applications, SBC has been shown to produce water of equal quality to conventional coagulation-flocculation-sedimentation processes (Desjardins *et al.*, 2002; Plum *et al.*, 1998). Although numerous

full-scale SBC units are in operation in Canada (Desjardins *et al.*, 2002), many of their performance claims are based on anecdotal/proprietary evidence as little data have been published in the peer reviewed literature on TOC and DOC removal at full-scale.

This study was undertaken to investigate the efficiency of NOM removal by SBC at a full-scale municipal DWTP. An advanced NOM characterization technique, liquid chromatography-organic carbon detection (LC-OCD), was used to determine the removal of specific method-defined NOM fractions through SBC (Huber *et al.*, 2011). NOM fractions characterized by LC-OCD include biopolymers, humic substances, and low molecular weight (LMW) compounds. The removal of biopolymers and humic substances, as quantified by LC-OCD, have been shown to be of particular importance due to their role in low pressure membrane fouling (Hallé *et al.*, 2009; Peldszus *et al.*, 2011; Tian *et al.*, 2013) and DBP formation (e.g. Wassink *et al.*, 2011), respectively. Traditional NOM parameters, including TOC and DOC, and humic substance indicators including ultraviolet absorbance (UVA) and specific ultraviolet absorbance (SUVA), were also evaluated through this study. While some information on humic substance reduction by coagulation is available using traditional quantification methods (e.g. Edzwald, 1993), little data are available for NOM fraction removal through SBC as determined by LC-OCD analysis.

4.3 Materials and Methods

4.3.1 Holmedale Water Treatment Plant

The study was conducted at the Holmedale Water Treatment Plant (HWTP) located in Brantford, Ontario, Canada. The treatment process consists of SBC (ACTIFLO^{TM}, Veolia Water Solutions & Technology; with whom no contact was made during this research project), ozonation, biofiltration, UV light disinfection, chlorination, and chloramination (Figure 4.1). The plant was designed to produce up to 100 mega litres of water per day (MLD), with average plant production of approximately 40 MLD throughout this study. The flow through the SBC process varied during the study, with a maximum raw water flow of 53 MLD in July 2012 and a minimum flow of 33 MLD in March 2013. Polyaluminum chloride (PACl) and polymer (MagnaflocTM LT27A, BASF, Germany, dose: 0.1-0.2 mg/L) were added in the SBC process at an average PACl dose of 33 mg/L (±7 mg/L), which varied with raw water flow and turbidity. Ozone was applied at a mean dose of 1 mg/L for the removal of taste and odour compounds, followed by dual-media biofiltration (1.6 m anthracite over 0.4 m sand). UV and chlorine contactors for primary disinfection followed the biofilters and finally ammonia was added to produce

monochloramine for secondary disinfection. The source water at the HWTP is the Grand River, which is the largest Canadian tributary of Lake Erie (Southam *et al.*, 1999). The Grand River watershed contains considerable agricultural activity, and several communities discharge treated sewage effluent upstream of the HWTP (Southam *et al*., 1999). The description of the complete process train has been provided only for context; SBC performance is the focus of this paper.

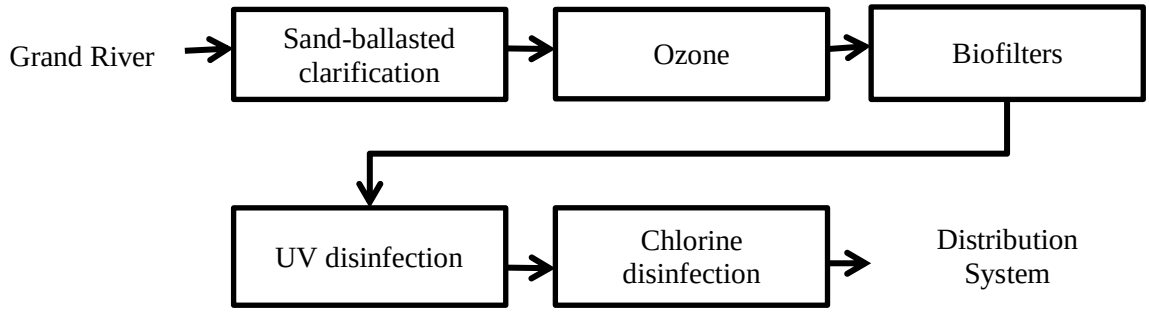


Figure 4.1: Holmedale Water Treatment Plant Process Diagram

4.3.2 Sample collection and analysis

Raw water and SBC effluent samples were collected twice per month from May 2012 to July 2013 at the HWTP. They were collected in clean 500 mL glass bottles and transported on ice from the HWTP to the laboratory (Waterloo, Ontario). Samples for DOC, LC-OCD and UVA at 254 nm (UVA_{254}) analyses were filtered through a pre-rinsed 0.45 μm polyethersulfone membrane (Pall Corporation, Mississauga, Ontario) within 24 h of collection. All samples were stored at 4˚C and analysed within one week of collection.

TOC and DOC concentrations were measured using a wet oxidation method with a Model 1010 Total Organic Carbon Analyser (O.I. Analytical, College Station, Texas) (Standard Methods, 2012, method 5310D). NOM fractions were measured by size exclusion chromatography using an LC-OCD together with ChromCALC software (DOC-LABOR, Karlsruhe, Germany) as described by Huber *et al.*, (2011). The optional correction factor that removes the LMW humics from the LMW acid fraction was disabled in the software.

Temperature was measured on site, and UVA_{254} and pH were measured in the laboratory. UVA_{254} was measured using a spectrophotometer (Hewlett Packard 8453) as described in Standard Methods (2012) method 5910 and pH was measured using an Orion pH meter (model 420A). The specific UV

absorbance (SUVA) was calculated by dividing the UVA_{254} (m^{-1}) by the DOC (mg/L) as is described in Standard Methods (2012) method 5910.

4.4 Results and Discussion

This study was undertaken to investigate the removal of NOM fractions at a full-scale drinking water treatment plant that employs SBC for particle and TOC removal. Determining the components in NOM can be challenging, however a unique NOM characterization technique, LC-OCD, was used to assess the ability of SBC to remove specific NOM fractions including biopolymers and humic substances, which can impact downstream DWTP processes. Biopolymers have been shown to contribute to hydraulically reversible fouling of low pressure membranes (Hallé *et al.*, 2009; Peldszus *et al.*, 2011; Tian *et al.*, 2013), while humic substances can contribute to the formation of DBPs (e.g. Wassink *et al.*, 2011). DBPs are formed when NOM reacts with oxidants used for disinfection in DWTPs, and although the health effects of many DBPs are still unknown, elevated concentrations over a long-term are potentially harmful (Crittenden *et al.*, 2012).

Between 2008 and 2012, prior to upgrades made at the HWTP aimed at increasing plant capacity, the average total THM (TTHM) concentration in the distribution system was 82 µg/L, compared with the Ontario maximum acceptable concentration for TTHMs of 100 µg/L (City of Brantford, 2013). After completion of the upgrades at the HWTP, which included the addition of ozonation, biofiltration and UV disinfection, TTHM levels decreased by 48% based on the 5 year average (City of Brantford, 2013). As part of a project to assess overall performance of the upgraded treatment plant, SBC was assessed to confirm its contribution to overall NOM removal. Turbidity removal efficiencies of SBC processes have previously been reported (e.g. Plum *et al.*, 1998), but this paper focuses on the dissolved organic carbon and NOM fraction removal efficiency of the SBC process.

4.4.1 TOC and DOC removal

Raw water quality and removal of TOC and DOC concentrations through SBC were monitored at the HWTP over the course of 14 months (Table 4.1). In the raw water, 97% of the TOC was in the dissolved form (Figure 4.2). SBC achieved average TOC and DOC removals of 30 and 32%, respectively (Figure 4.2). A paired t-test confirmed statistically significant removal of TOC and DOC through SBC over the study period at the 99% confidence level ($p<0.01$). TOC and DOC removals through SBC were consistent through the year, and did not exhibit any seasonal trends (Figure 4.3).

Table 4.1 Grand River water quality, May 2012 to July 2013

Water quality parameter	Unit	Mean	Standard deviation	n
TOC	mg C/L	6.31	0.45	22
DOC	mg C/L	6.10	0.38	22
pH	-	8.1	0.3	22
Temperature	˚C	16	9	22
UVA_{254}	cm^{-1}	0.165	0.017	22
SUVA	L/mg C•m	2.69	0.17	22
Turbidity*	NTU	13.23	10.44	457
Alkalinity*	mg/L $CaCO_3$	196	27	73

* Data from the City of Brantford.

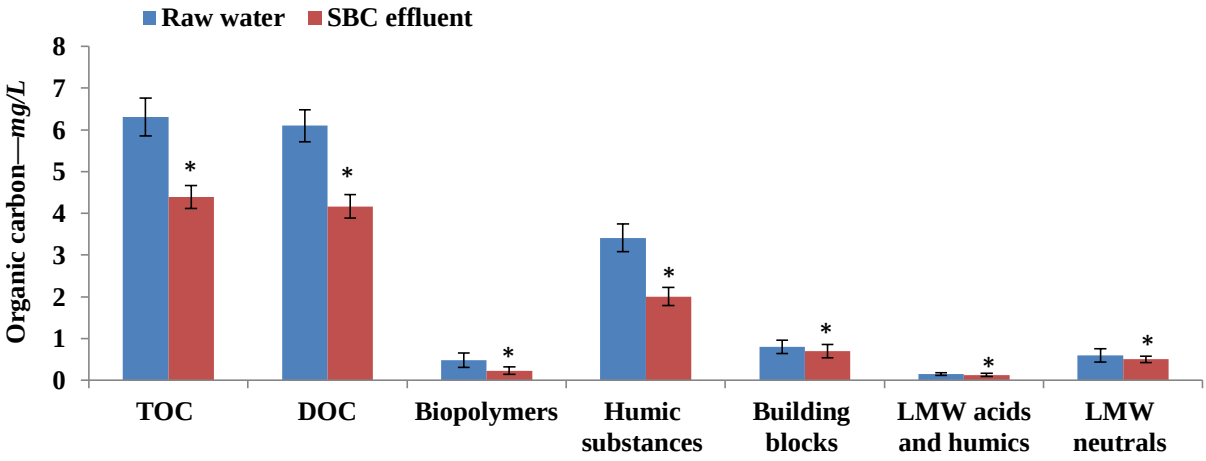


Figure 4.2 Organic carbon concentrations through SBC at the HWTP (n=22). Error bars show the standard deviation *shows statistically significant removal through SBC (p<0.01).

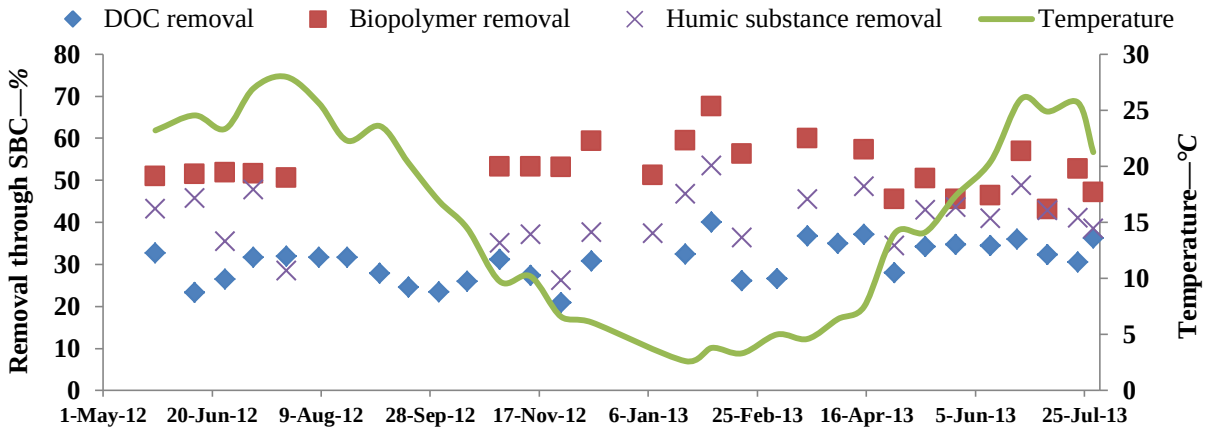


Figure 4.3 Organic matter removal through SBC at the HWTP

At the HWTP, substantial TOC and DOC removal was achieved through SBC without intentional pH adjustment. The average pH of the raw water was only reduced from 8.1 to 7.6 after coagulant addition in the SBC process and appears to vary minimally with coagulant dose (Figure 4.4). The average pH after coagulation was well above the suggested optimal pH range for enhanced coagulation of between 5.5 and 6.5 (USEPA, 1999). The average TOC removal of 30% achieved through SBC was greater than the required TOC removal of 25% (based on raw water TOC and alkalinity values) stipulated by the United States National Primary Drinking Water Regulations: Disinfectants and Disinfection Byproducts (USEPA, 1998). The USEPA requires TOC removal to be achieved through enhanced coagulation in plants using conventional treatment, including coagulation, flocculation or precipitative softening, sedimentation and filtration (USEPA, 1998).

Prior to the installation of the SBC process, the HWTP used conventional coagulation (with alum and activated silica) at pHs between 7.0 and 7.2, and achieved 9% nonpurgeable organic carbon (NPOC) removal (Huck *et al.*, 1994). NPOC is the organic carbon fraction that remains in a water sample after acidification and sparging to remove inorganic carbon, and is equivalent to TOC quantified in this study. Urfer *et al.* (1999) showed that for the Grand River, NPOC is composed of approximately 90 to 95% DOC. Since the Huck *et al.* (1994) study was performed, numerous changes have occurred at the HWTP which may have contributed to increased TOC removal, including changes in the type of coagulant and in activated silica use, to name a few. For this reason, a direct comparison between conventional coagulation and SBC at the HWTP cannot be performed.

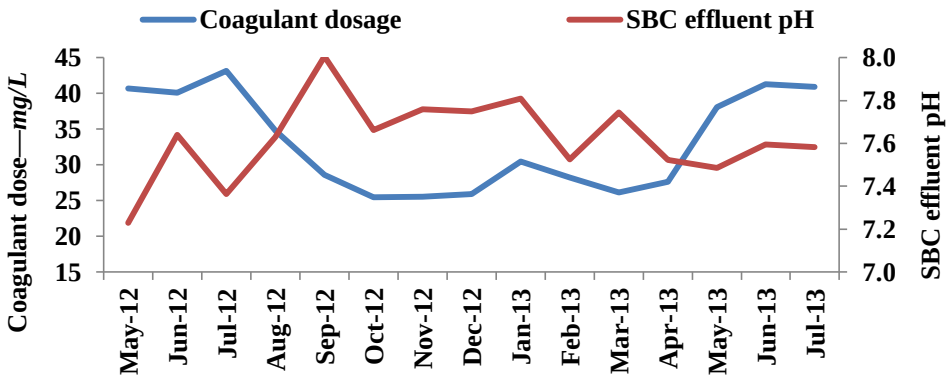


Figure 4.4 Coagulant dose and pH through SBC at the HWTP

Studies have been performed at a DWTP upstream of the HWTP which also uses the Grand River as source water. This plant differs from the HWTP in that the treatment processes are preceded by several days of raw water storage (Urfer *et al.*, 1999). In addition, this plant uses conventional coagulation (with PACl and polymer) rather than SBC, at a similar pH as the HWTP (pH 7.7). DOC removal through conventional coagulation at this plant was reported as 29% (Croft, 2014), which was similar to the removal through the HWTP SBC (32%). Earlier studies at a DWTP upstream of the HWTP by Urfer *et al.* (1999) reported approximately 10% NPOC removal through conventional coagulation (using alum and polymer) at pH values between 6.91 and 7.20, and 28% NPOC removal through enhanced coagulation (using alum, polymer, and pH suppression) at pH values between 6.0 and 6.3.

In studies of other DWTPs that use SBC processes, Rodriguez *et al.* (2007) reported TOC removal of 31% through a full-scale SBC process at pH 6.51, treating river water with pre-chlorination employed. Croft (2014) also reported 32% DOC removal through sand-ballasted assisted flocculation using acidified alum, polymer, and silicate (average pH 7.3) at a full-scale municipal DWTP treating water from Lake Huron, Ontario, Canada.

4.4.2 NOM fraction removal

The various operationally-defined NOM fractions quantified by LC-OCD include biopolymers (compounds with molecular weight greater than 10 kDa), humic substances, building blocks (breakdown products of humic substances), LMW acids and humics, and LMW neutrals (including LMW alcohols, aldehydes, ketones, sugars and amino acids), as described by Huber *et al.* (2011). Results show that humic substances made up the largest part (56%) of DOC in raw Grand River water (Figure 4.2). Biopolymers, building blocks, LMW acids and humics, and LMW neutrals accounted for

8%, 13%, 2%, and 10% of the raw water DOC, respectively. The results for humic substances are in line with others who have found that humic substances typically represent 40 to 60% of the total DOC in natural waters (Thurman, 1985).

The SBC process contributed to statistically significant biopolymer and humic substance removals of 53% and 41%, respectively (p<0.01) (Figure 4.2). Humic substances were the NOM fraction that was removed by the largest amount through SBC, with average raw water humic substance concentrations of 3.41 mg/L that were reduced to 2.01 mg/L in the SBC effluent. The SBC process also achieved statistically significant removal of the other NOM fractions, including 12% building blocks, 16% LMW acids and humics, and 16% LMW neutrals. The removal of DOC and most NOM fractions through SBC did not exhibit any seasonal variations (Figure 4.3), with the exception of building blocks and LMW neutrals (data not presented). The removal of DOC, biopolymers, and humic substances were not correlated to coagulant dose (Figure 4.5), or SBC pH (data not presented for reasons of space).

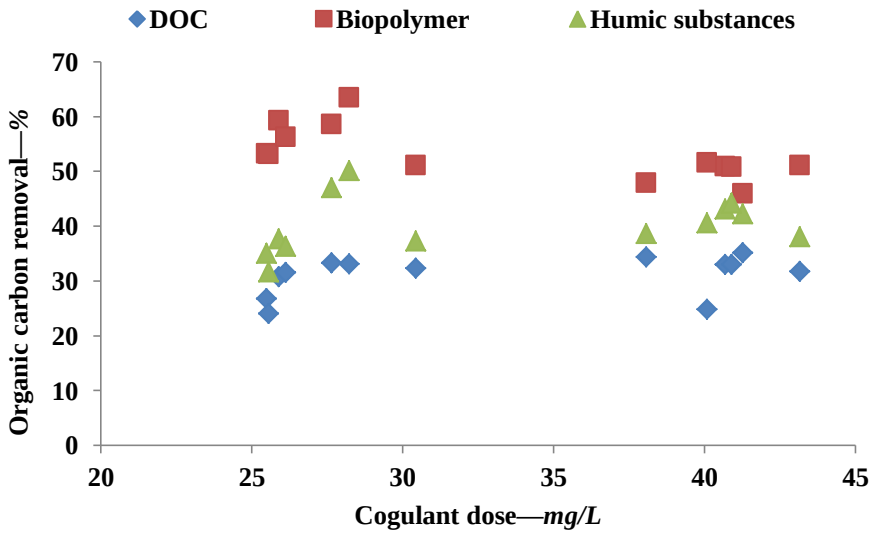


Figure 4.5 Organic carbon removal vs. coagulant dose (n=13)

The removal of biopolymers and humic substances through SBC was similar to that reported through conventional coagulation (using PACl and polymer) at a DWTP located upstream of the HWTP, with 56% biopolymer removal and 37% humic substance removal at pH 7.7 (Croft, 2012; Croft, 2014). In addition, similar biopolymer and humic substance removals were reported at a full-scale DWTP treating Lake Huron, Ontario water, where 61% biopolymer and 34% humic substance removals were measured through sand-ballasted assisted flocculation using acidified alum, polymer and silicate (average pH 7.3)

(Croft, 2012; Croft, 2014). Results from the same study found that enhanced coagulation (using alum, silicate, and pH suppression) at pH 5.8 of Ottawa River water was able to achieve similar biopolymer removal (57%) compared to the HWTP, while humic substance removal (67%) was higher (Croft, 2012). Recognizing that the water types are different, it is possible that the removal of humic substances may be more affected by pH suppression than is biopolymer removal. This may be due to the high charge density of functional groups in humic acids, which at reduced pH makes them more susceptible to removal through charge neutralization (Croft, 2012; Owen *et al.*, 1995). The considerable removal of biopolymers through SBC is important in terms of hydraulically reversible low pressure membrane fouling, and decreased biopolymer concentrations would result in reduced membrane run times between backwash and cleaning cycles (Hallé *et al.*, 2009; Peldszus *et al.*, 2011; Tian *et al.*, 2013).

In addition to NOM fraction analysis by LC-OCD, UVA_{254} and SUVA can also provide information relating to the concentration of NOM and specifically humic substances in water. UVA_{254} is a useful indicator of the presence of aromatic compounds in water, including humic substances, due to their strong absorption of UV radiation (Standard Methods, 2012). A linear relationship was found between UVA_{254} and the DOC concentration in raw and SBC effluent water (Figure 4.6). These results are similar to others who have also reported a linear relationship between DOC/TOC and UVA_{254} (e.g. Wassink *et al.*, 2011). The line between DOC and UVA_{254} does not pass through the origin as there are some organic compounds that do not absorb UV light, such as aliphatic acids, alcohols, and sugars (Edzwald *et al.*, 1985). Study results from the HWTP showed that SBC was able to reduce the mean UVA_{254} from 0.165 cm^{-1} in the raw water to 0.092 cm^{-1} in the SBC effluent, resulting in a 44% reduction (Table 4.2). UVA_{254} removal was similar to the mean humic substances removal (41%) calculated using LC-OCD data (Table 4.2). Croft (2014) similarly measured 40% UVA_{254} reduction through conventional coagulation (using PACl and polymer) at a DWTP location upstream of the HWTP on the Grand River. Volk *et al.* (2000) also reported similar levels of UVA_{254} reduction, 40 and 33%, through conventional coagulation (using PACl) at two different DWTPs with similar raw water TOC and alkalinity as the HWTP. The study also found that enhanced coagulation using pH suppression increased UVA_{254} removal to 65 and 52% at the two plants (Volk *et al.*,2000). Croft (2014) also found that a DWTP that used enhanced coagulation (using alum, silica and pH suppression) of Ottawa River water resulted in high reduction (74%) of UVA_{254}. Overall, the reduction of UVA_{254} at the HWTP falls within the range of values reported by others through conventional coagulation (using alum and PACl). Further comparisons with published data indicate that greater UVA_{254} or humic substances reduction can only be achieved using enhanced coagulation with pH suppression

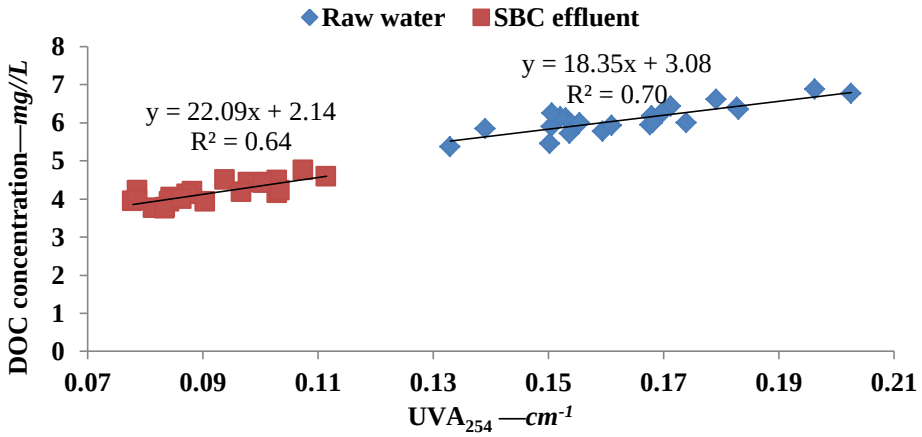


Figure 4.6 DOC vs. UVA_{254} in raw water and SBC effluent (n=22)

SUVA is a ratio of the UV absorbance and DOC concentration, and provides some information about the proportion of aromatic, mostly humic, content of the organic matter in a given water source (Edzwald, 1993; Edzwald & Tobiason, 1999). Although a substantial UVA_{254} reduction was observed through SBC at the HWTP, the SUVA value was only reduced by 0.44 L/mg C•m (18%) (Table 4.2). These results demonstrate that, unlike UVA_{254}, the removal of humic substances, as quantified by LC-OCD, through SBC was not related to SUVA reduction (Table 4.2). Urfer *et al.* (1999) also reported similar results for SUVA reduction through enhanced coagulation (using alum, polymer and pH suppression) of Grand River water at a DWTP located upstream of the HWTP (coagulation pH between 6.15 and 7.05). Their results showed that the approximate SUVA value, calculated by dividing the UVA_{254} by the NPOC concentration, was reduced from 3.6 L/mg•m in the raw water to 3.0 L/mg•m after enhanced coagulation, flocculation, sedimentation, resulting in a 17% reduction (Urfer *et al.*, 1999). Croft (2014) also reported 16% SUVA reduction through conventional coagulation (using PACl and polymer) at a DWTP located upstream of the HWTP. Similarly, Croft (2012) reported no direct correlation between humic substance removal, as quantified by LC-OCD, and SUVA reduction through coagulation at numerous other full-scale water treatment plants. Ho *et al.* (2013) also reported that treatment processes that reduced both UVA_{254} and DOC showed little change in SUVA. Since SBC results in a reduction of both UVA_{254} and DOC, it is reasonable that the ratio of these two values would be less affected than UVA_{254} alone.

Table 4.2 Mean UVA_{254}, SUVA and humic substances (by LC-OCD) concentrations and their reduction through SBC

	Raw Mean (s.d.)	SBC effluent Mean (s.d.)	Mean reduction through SBC (%)
Humic substances (mg/L) (n=22)	3,41 (0,33)	2,01 (0,22)	41
UVA_{254} (cm^{-1}) (n=22)	0,165 (0,017)	0,092 (0,010)	44
SUVA (L/mg C•m) (n=22)	2,69 (0,17)	2,20 (0,15)	18

LC-OCD provides valuable information about the NOM fractions in water; however, there are little data on its relationship with conventional water quality parameters such as UVA_{254}. Results from this study show a strong correlation for both raw and SBC effluent water between the concentration of humic substances, determined by LC-OCD and UVA_{254} (Figure 4.7). The line between humic substances and UVA_{254} does not pass through the origin, possibly because the absorptivity of all humic substances is not equal, resulting in UVA_{254} variations (Her *et al.*, 2002). Results demonstrate that the relationship between humic substance concentration, as determined by LC-OCD and UVA_{254} is the same in the raw water and following the SBC process.

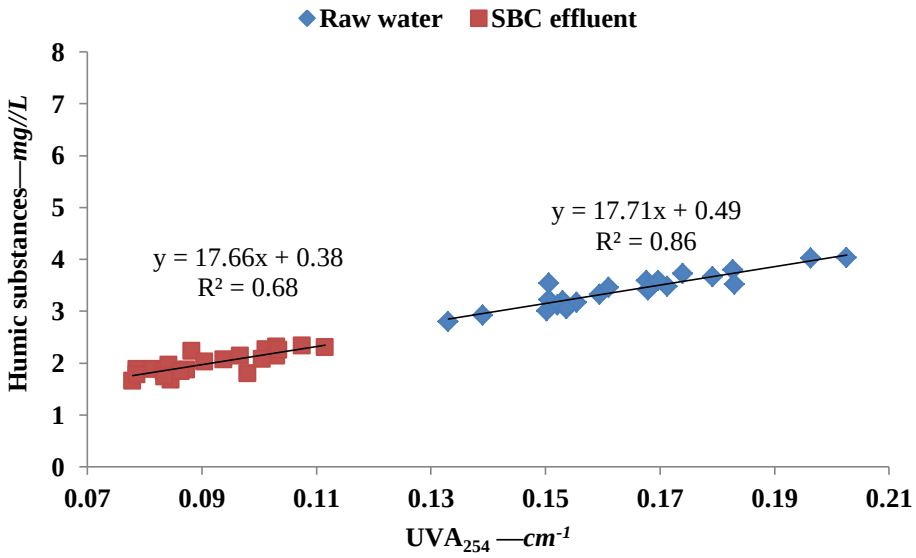


Figure 4.7 Humic substances vs. UVA_{254} in raw water and SBC effluent (n=22)

4.5 Conclusion

Under the conditions investigated, this 14 month study demonstrates sand-ballasted clarification (SBC) to be an effective process for NOM removal with substantial TOC and DOC removals of 30 and 32%, respectively. However, due to limited historical data and the use of different coagulants in various studies, the performance of SBC at the plant being investigated could not be directly compared to that of other coagulation processes. LC-OCD provided valuable information relating to the removal of specific NOM fractions through SBC, and in particular provided data on biopolymer and humic substance removal which can impact other DWTP processes and finished water quality. In this investigation, SBC removed significant concentrations of higher molecular weight NOM fractions, including 53% of the biopolymers (proteins, polysaccharides, etc.), and 41% of the humic substances. Although the HWTP does not use membranes as part of their current operations, the ability of SBC to remove a high level of biopolymers will be valuable for future infrastructure planning and will also be of interest to other plants that use membranes. Humics substance removal as measure by LC-OCD was similar to the UVA_{254} reduction of 44%. Results also show that the character of the DOC and humic substances remains unchanged through SBC, and that a similar relationship exists in both raw and SBC effluent water between the concentrations of DOC, humic substances and UVA_{254}. The work presented in this study will contribute to a greater understanding of the important factors that contribute to the removal of specific NOM fractions by coagulation.

4.6 Disclaimer

The authors have had no contact or affiliation with Veolia Water Solutions & Technologies and their Canadian subsidiaries, nor did those companies provide any financial incentives in the form of products or materials. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Chapter 5
Seasonal Performance of Full-Scale Ozone-biofiltration for NOM Component Removal

5.1 Overview

The removal of carbon and nitrogen fractions of natural organic matter (NOM) through drinking water treatment processes is important to, among other things, reduce the concentration of disinfection by-product precursors. One process known to remove such fractions is ozone-biofiltration. Due to the potential effects seasonal variations in water temperature and quality can have on this process, the efficiency of full-scale ozone-biofiltration over 14 months was investigated. Results indicated significant raw water fluctuations in certain NOM fractions (biopolymers) and nitrogen-containing compounds, such as total nitrogen, nitrate and ammonia. The performance of the ozone process was unaffected by temperature, while the biofilters exhibited reduced removal of dissolved organic carbon (DOC), NOM fractions (biopolymers and low molecular weight acids and humics), and assimilable organic carbon at T≤10°C. No significant removal of nitrogen-containing compounds was observed through ozone-biofiltration during the present study. Although the biofilters exhibited reduced performance at T≤10°C, the quantity and activity of the biomass in the biofilters remained constant, and overall the water treatment plant was able to achieve significant removals of all NOM compounds.

5.2 Introduction

Natural organic matter (NOM) is a complex matrix of compounds, predominantly composed of carbon, roughly 40 to 60% by weight, and contains a small fraction of nitrogen, 1 to 5% by weight (IHSS, 2013; Lee & Westerhoff, 2006). The removal of NOM in drinking water treatment is important and can be challenging for drinking water providers. The carbon fraction of NOM, typically approximated by dissolved organic carbon (DOC), has been shown to provide precursors to disinfection by-products (DBPs), increase disinfectant demand and contribute to microbial regrowth and corrosion in distribution systems (Jacangelo *et al.*, 1997). The nitrogen fraction of NOM, which can be approximated by dissolved organic nitrogen (DON), reacts with disinfectants to form N-nitrosodimethylamine (NDMA) and more recently several other newly identified carcinogenic nitrogenous DBPs (Lee & Westerhoff, 2006). The presence of inorganic nitrogen species in drinking water, such as nitrate and nitrite, are also known to cause serious health effects in infants at elevated concentrations (Kapoor & Viraraghavan,

1997). Other nitrogenous compounds (e.g. ammonia) will react with free chlorine to produce chloramines reducing chlorine disinfection efficacy. For these reasons, the removal of elevated levels of both the carbon and nitrogen fractions of NOM through drinking water treatment processes is of importance.

The use of advanced water treatment processes, such as ozone followed by biofiltration (ozone-biofiltration), has become widespread due to the numerous benefits these processes provide. Ozone acts as an oxidant and, among other effects, degrades a fraction of NOM into biodegradable organic matter (BOM), which is predominantly comprised of low molecular weight compounds (Volk & LeChevallier, 2002, Volk *et al.*, 1993). The BOM produced during ozonation can then be partially removed through biofiltration, where it is used by the biomass as a source of energy and carbon (Volk & LeChevallier, 2002). Coupled ozone-biofiltration processes have been shown to reduce the formation of regulated DBPs (Yan *et al.*, 2010), decrease disinfectant demand, remove taste and odour causing compounds (Nerenberg *et al.*, 2000), increase the biostability of finished water, and lead to reduced regrowth within distribution systems (Price *et al.*, 1993). Additionally, studies have shown that under the right conditions, ozone-biofiltration can remove DON, nitrogenous DBP precursors (Chu *et al.*, 2012), and ammonia (Wert *et al.*, 2008).

Although numerous studies have evaluated the efficiency of ozone-biofiltration, few have investigated the effects of seasonal changes on the efficiency of this process at full-scale. Such an investigation is of great importance, as temperature has been shown to impact biofilter performance (Huck & Sozański, 2008), and in the present study seasonal temperature fluctuations in the raw water varied between a low of 3°C and a high of 28°C. In addition, a limited number of studies have utilized advanced NOM characterization techniques, such as liquid chromatography-organic carbon detection (LC-OCD), to measure NOM removal through full-scale ozone-biofiltration processes. Such determination is important as previous studies have identified that certain operationally defined NOM fractions, such as biopolymers and humic substances quantified by LC-OCD, can contribute to fouling of low pressure membranes (Hallé *et al.*, 2009) and can act as precursors to DBPs (Wassink *et al.*, 2011). As such, the objectives of the present study were to investigate the effects of seasonal variations on full-scale performance of ozone-biofiltration. Ozone-biofiltration performance for the removal of different NOM fractions was quantified over a 14 month period using a range of analyses, including LC-OCD, assimilable organic carbon (AOC), and investigation into specific nitrogen compounds including total nitrogen, nitrate and ammonia. Seasonal variations in the viable biomass quantity and

activity within the biofilters were also determined using adenosine triphosphate (ATP) and fluorescein diacetate (FDA) hydrolysis methods.

5.3 Materials and Methods

5.3.1 Holmedale Water Treatment Plant (HWTP)

The source water at the HWTP is the Grand River, and due to considerable agricultural activity in the watershed substantial fluctuations in raw water quality are experienced throughout the year (Southam *et al.*, 1999). The treatment process at the HWTP consists of sand ballasted clarification (SBC) (ACTIFLO^{TM}), ozone for taste and odour removal, biofiltration, ultraviolet (UV) light for primary disinfection, chlorine for primary disinfection, and chloramination for secondary disinfection (Figure 5.1). The plant was designed to produce up to 100 mega litres of water per day (MLD), with average plant production during the present study of 40 MLD. A mean dose of 33 mg/L of polyaluminum chloride was applied prior to the SBC process along with a polymer (Magnafloc^{TM} LT27A, BASF, Mississauga, Canada). The mean applied ozone dose was 1 mg/L and the process was operated to ensure that no ozone residual reached the biofilter influent. Following ozonation, there are eight dual media, anthracite over sand, biofilters which were operated with an average empty bed contact time (EBCT) of 38 minutes (at 40 MLD). The biofilters each contained 1.6 m of anthracite (effective size [ES] 1.0-1.2 mm, specific gravity [SG] 1.4) over 0.4 m of sand (ES 0.35-0.45 mm, SG 2.65). Backwashing of the biofilters was done with non-chlorinated water, and included air scour, low wash (400 L/s) and high wash (800 L/s). Finally, UV and chlorine were used for primary disinfection followed by chloramination for secondary disinfection. During the present study, the applied UV dose was 20 mJ/cm^2, and the average free chlorine residual in samples taken at the end of the contact chambers was 2.76 mg/L.

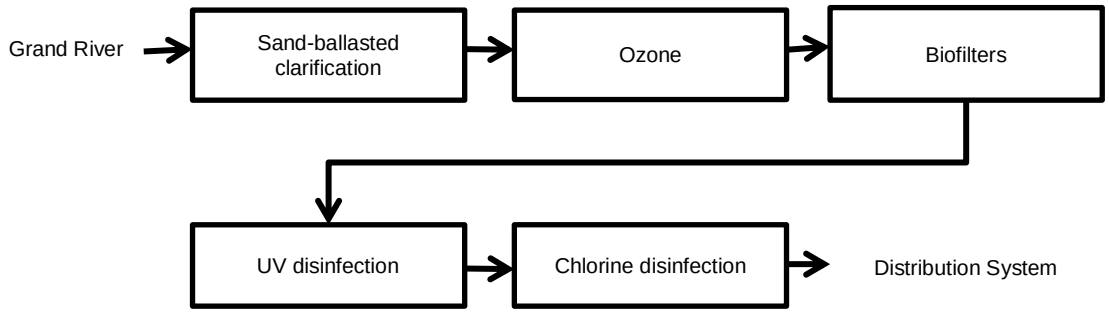


Figure 5.1: Holmedale Water Treatment Plant Process Diagram

5.3.2 Sample collection

From May 2012 to July 2013, water samples were collected twice per month (n = 31) at the HWTP in clean glass bottles from the following locations: raw water, SBC effluent, ozone effluent, biofilter effluent, and chlorine effluent prior to chloramination. In addition, anthracite media samples (n = 29) from an operational biofilter were collected in 50 mL sterile polypropylene centrifuge tubes from the top 5-10 cm of the biofilter using an extension pole. The surface of the biofilter was first scraped away to allow sampling below the top layer. Water and media samples were transported on ice to the laboratory (Waterloo, Ontario).

5.3.3 Water quality analysis

All laboratory analyses were performed at the University of Waterloo. Samples for DOC, LC-OCD and ultraviolet absorbance at a wavelength of 254 nm (UVA_{254}) were filtered through pre-rinsed 0.45 μm polyethersulfone membranes (Pall Corporation, Mississauga, Ontario) within 24 hours of sample collection and stored at 4˚C until analysis. DOC was quantified by wet oxidation using a Model 1010 Total Organic Carbon Analyser (O.I. Analytical, College Station, Texas) and samples were preserved with phosphoric acid to a pH ≤2 on the day of sample collection (Standard Method 5310D [2012]). LC-OCD (DOC-LABOR, Karlsruhe, Germany) was used to quantify operationally defined NOM fractions and consists of size exclusion chromatography (Huber *et al.*, 2011). The NOM fractions quantified include: biopolymers (compounds with molecular weight greater than 10 kDa, such as polysaccharides and proteins), humic substances, building blocks (breakdown products of humic substances), low molecular weight (LMW) acids and humics, and LMW neutrals (including low molecular weight alcohols, aldehydes, ketones, sugars and amino acids), as defined and described by Huber *et al.* (2011). ChromCALC software was used to determine the concentration of the specific NOM fractions present in each sample (Huber *et al.*, 2011). The automated correction that excludes the LMW-humics which co-elute with the LMW-acids was not used, and therefore this fraction included both LMW-acids and humics. The method detection limits for the LC-OCD quantified biopolymers, humic substances, building blocks, and LMW neutrals were 9 μg/L, 9 μg/L, 26 μg/L, and 44 μg/L, respectively. The minimum reporting levels for the biopolymers, humic substances, building blocks, and LMW neutrals were 26 μg/L, 26 μg/L, 77 μg/L, and 131 μg/L, respectively. AOC was quantified according to Standard Method 9217B (2012) in SBC effluent, ozone effluent and biofilter effluent samples and AOC analysis was started within 24 hours of sample collection. Briefly, this method measures the regrowth of *Pseudomonas fluorescens* strain P-17 (ATCC 49642) and *Spirillum sp*. strain NOX (ATCC 49643) in a

sample using a spread plate technique onto R2A agar (BD, Sparks, Maryland), and the amount of regrowth is then converted to an acetate equivalent carbon concentration.

Total nitrogen was quantified using HACH method 10071 (persulfate digestion) and had a detection limit of 0.05 mg N/L. This method measures both organic and inorganic forms of nitrogen. Nitrate was measured using HACH method 8171 (cadmium reduction), and had a detection limit of 0.1 mg NO_3—N/L. Ammonia was determined using HACH method 10023 (salicylate) and had a detection limit of 0.02 mg NH_3-N/L. All nitrogen containing compounds were analysed on a HACH DR 2500 spectrophotometer (HACH, Colorado, United States). Temperature was measured on site at the time of sample collection. UVA_{254} measurements were done using a Hewlett Packard 8453 spectrophotometer according to Standard Methods (2012) method 5910.

5.3.4 Biomass quantity and activity

ATP was used to determine the quantity of biomass, and was measured using the LuminUltra Deposit & Surface Analysis kit (LuminUltra, Fredericton, Canada). ATP concentrations are presented in ng ATP/cm^3 media, and were determined using a bulk density of 0.67 g dry weight/cm^3 for anthracite. Biomass activity was monitored by FDA hydrolysis using a similar method as described by Seredyńska-Sobecka *et al.* (2006). Briefly, 1 mg wet weight of biofilter media was mixed with 50 mL of 60 mM sodium phosphate buffer (pH 7.6) and 0.5 mL of 4.9 mM FDA substrate (20 mg FDA [Calbiochem, EMD Millipore, Massachusetts, United States] in 10 mL acetone [Fisher-Scientific, Ottawa, Canada]). The sample was incubated for 3 hours at 37°C without mixing. After incubation, 2 mL was removed and centrifuged for two minutes in a sterile microcentrifuge tube, and the absorbance was measured at a wavelength of 490 nm using a Hewlett Packard 8453 spectrophotometer. A blank made of 50 mL sodium phosphate buffer and 0.5 mL acetone without biofilter media was used. To develop a standard curve, a 0.6 mM fluorescein stock solution (11.3 mg Na_2-fluorescein [Sigma-Aldrich, Oakville, Canada] in 50 mL of 60 mM sodium phosphate buffer [pH 7.6]) was used to prepare standards of the following concentrations: 0, 30, 100, 300 and 500 µg fluorescein per 50 mL.

5.3.5 Statistical analysis

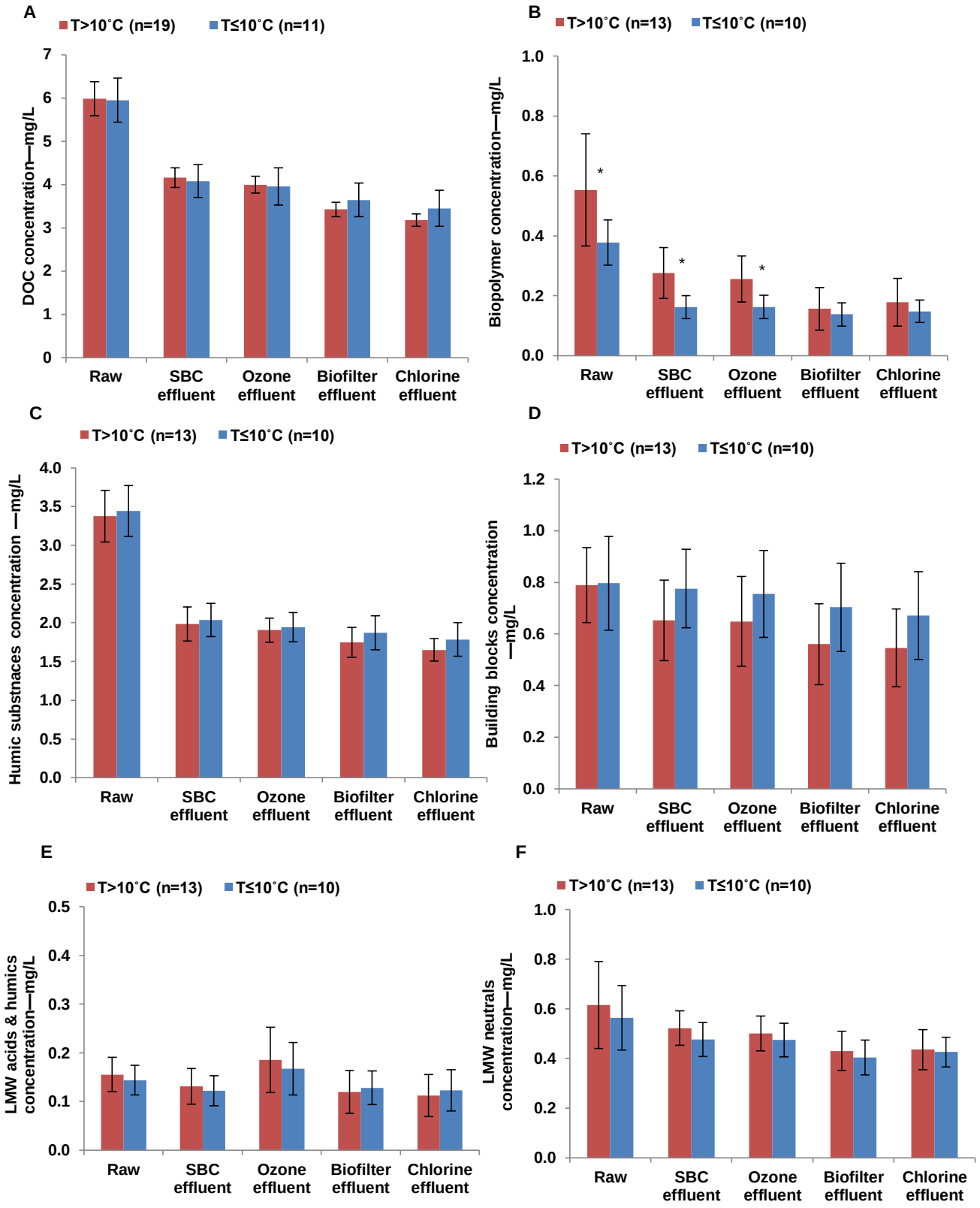
For the purpose of statistical analyses, data below the detection level were assigned a value of 50% of the detection limit (USEPA, 2000). The effect of seasonal variations in raw water quality on process performance were investigated by comparing periods when the raw water temperature was greater than 10°C ($T>10°C$) and when it was less than or equal to 10°C ($T\leq10°C$). The cut-off of 10°C was also

used in a previous study to assess pathogen concentration in the Grand River, and was selected since it was close to the average temperature in the Grand River (11°C) (Van Dyke *et al.*, 2010). Student's t-tests (two tailed) were used to determine statistical differences between temperature ranges. Paired t-tests (two tailed) were used to determine if the difference in removal through a specific treatment process at the HWTP was statistically significant. For both the student t-test and the paired t-test, $\alpha = 0.05$ was used to determine statistical significance.

5.4 Results and Discussion

5.4.1 NOM fraction concentration and removal

During the 14 month investigation, results showed that the biopolymer concentration varied significantly in the raw river water with seasonal temperature changes, from 0.55 mg/L at T>10°C to 0.38 mg/L at T≤10°C (Figure 5.2B). The concentration of DOC and all other NOM fractions in the raw water did not change significantly with temperature (Figure 5.2). The average raw water DOC concentration was 5.97 mg/L (n=30), humic substance concentration was 3.40 mg/L, building blocks was 0.79 mg/L, LMW acids and humics was 0.15 mg/L, and LMW neutrals was 0.59 mg/L (n=23). These biopolymer results are similar to those reported by others, such as Hallé (2009) who observed higher concentrations of biopolymers, quantified by LC-OCD, in the Grand River during summer months. Croft (2012) investigated the seasonal variation in biopolymers, quantified by LC-OCD, in six water sources, including both lakes and rivers, and also reported that overall there were higher concentrations of biopolymers observed in the warmer summer months. Similar to the results



*—statistically significant difference in concentration ($p<0.05$) between T>10°C and T≤10°C.

Figure 5.2: Mean NOM concentrations through the HWTP at water temperatures (T) >10°C and T≤10°C. Error bars represent ± standard deviation.

presented above, Croft (2012) also reported no significant seasonal fluctuations in the concentration of humic substances in the six water sources investigated. Croft (2012) indicated that higher biopolymer concentrations at warmer temperatures is likely due to the greater microbiological activity in water at warm temperatures, which leads to the production of a greater concentration of proteins and polysaccharides. It is also speculated that increased agricultural activity during summer months, along with increased growth of vegetation, and presence of birds and animals in the watershed, could also lead to increased concentrations of biopolymers in the Grand River during warm weather periods.

At the HWTP raw water passes through SBC prior to the ozone contactor. The performance of the SBC process for NOM removal was discussed in more detail in a previous chapter (Chapter 4), but these data are also presented in this chapter to provide information on the ozone-biofilter feed water and overall NOM removal by the plant. SBC was previously reported (Chapter 4) to remove high amounts of DOC (32%), biopolymers (53%) and humic substances (41%), and somewhat lower removal of building blocks, and low molecular weight compounds (12 to 16%). Seasonal differences in NOM removal by SBC were observed only for the building blocks and LMW neutrals fractions (Table 5.1). Therefore, prior to ozonation a considerable fraction of high molecular weight fractions were removed through SBC.

Table 5.1: Effect of seasonal changes and treatment performance on NOM fraction removal at the HWTP

Temperature (°C)		DOC		Biopolymers		Humic substances		Building blocks		LMW acids & humics		LMW neutrals	
		>10 (n=19)	**≤10** (n=11)	**>10**	**≤10**	**>10**	**≤10**	**>10**	**≤10**	**>10**	**≤10**	**>10**	**≤10**
Mean % removal	**SBC**	31†	31†	50†	57†	41†	41†	**17†**	**3**	16†	15†	**15**	**15†**
	Ozone	4†	3†	**7*†**	**0***	4†	5†	1	3	-42†	-38†	**4†**	**0**
	Biofilter	14*†	8*†	39*†	15*†	**8†**	**4**	14†	7†	36*†	24*†	14†	15†

*****—statistically significant difference in removal (p<0.05) between T>10°C and T≤10°C.
†—statistically significant removal (p<0.05) from previous treatment step.
bold— NOM fraction removal is not statistically significant in both temperatures ranges.
For T >10 n=13 and for T ≤10 n=10, unless otherwise indicated.
DOC—dissolved organic carbon, LMW—low molecular weight, SBC—sand-ballasted clarification.

5.4.1.1 Ozonation

The ozone process at the HWTP, designed for taste and odour removal, resulted in low DOC removal and no difference in removal with temperature, with 4% and 3% removals reported at T>10°C and

T≤10°C, respectively (Table 5.1). The ozone process contributed to statistically significant biopolymer removal at T>10°C (7% removal), while the process did not remove biopolymers at T≤10°C (0% removal) (Table 5.1). The difference observed in biopolymer removal through ozone at different temperatures may be due to seasonal differences in the character and concentration of biopolymers. For the other NOM fractions, removal by ozone did not change with temperature. The ozone process resulted in a statistically significant reduction in the humic substance fraction with an overall removal of 4% (0.09 mg/L), and a statistically significant increase in LMW acids and humics of 40% (0.05 mg/L) overall. However there was little overall removal of building blocks (2%) and LMW neutrals (3%). The effect of ozone on certain NOM fractions was expected as it is known that ozone oxidizes organic compounds and produces more easily BOM, which tends to be smaller in size (Volk & LeChevallier, 2002, Volk *et al.*, 1993). These smaller compounds may relate in particular to the increase in LMW acids and humics observed through ozone in the present study. Vasyukova *et al.* (2013) reported that ozone, at an applied ozone dose of 0.65 mg O_3/L, had no impact on the concentration of any NOM fractions, as quantified by LC-OCD, when investigating a full-scale water treatment plant treating organic-rich surface water. However, Croft (2012) also reported an increase in the LMW acid and humic fraction, as quantified by LC-OCD, following ozone in a different drinking water treatment plant that also uses the Grand River as source water.

The effect of ozone on the concentration and character of aromatic compounds was also investigated by measuring UVA_{254}, as aromatic compounds, such as humic substances, strongly absorb ultraviolet radiation (Standard Methods, 2012). Through the ozone process, the average UVA_{254} was reduced from 0.089 cm^{-1} to 0.058 cm^{-1} (n=31), which represents a 35% removal. Ozonation resulted in a much higher percent reduction in UVA_{254} compared to humic substances as quantified by LC-OCD. This is likely because ozone changes the structure and reduces the aromatic humic compounds, while their size may be less affected and therefore remains in the fraction quantified as humic substances by LC-OCD.

5.4.1.2 Biofiltration

Following ozonation, the biofilters achieved considerable removal of DOC and the different NOM fractions, including on average 12% DOC (n=30), 31% biopolymers, 6% humic substances, 10% building blocks, 31% LMW acids and humics and 14% LMW neutrals (n=23). The high removal of biopolymers, and LMW acids and humics through the biofilters suggests that these fractions may be more biodegradable than the other LC-OCD fractions. The biofilters were able to reduce the LMW acid and humic concentration down to pre-ozonation levels, which points to the good performance of the

ozone-biofilter process at the HWTP. The removal of LMW acids and humics following ozonation in drinking water treatment is of particular importance as this fraction may contribute to increased organic carbon availability which may lead to biofouling of certain membranes (Huck & Sozański, 2008), such as high-pressure membranes in which the impact of biofilm formation on membrane performance has been shown to be strong (Dreszer *et al.*, 2014). Biofilter NOM fraction removal was previously investigated in a full-scale water treatment plant treating organic-rich water in Germany, and reported that GAC biofilters, preceded by coagulation, sand filtration, and ozonation were able to achieve 35% DOC removal, 25% building blocks removal, and 50% LMW neutrals removal (Vasyukova *et al.* 2013). In their study, LMW acids were below the detection limit in all samples, and the removal of biopolymers and humic substances through the biofilters were not reported (Vasyukova *et al.*, 2013). Another study performed at a full-scale water treatment plant also treating Grand River water reported that biofilters preceded by coagulation and ozonation achieved between 0 and 37% biopolymer removal, between 0 and 15% humic substance removal, 10% building block removal, 84% LMW acid removal, and 17% LMW neutral removal (Croft, 2012; 2014). These results demonstrate that there is high variability in NOM fraction removal rates between different studies on full-scale biofilters after ozonation, and suggest that raw water NOM fraction concentrations and treatment processes upstream of the biofilters (such as SBC) can greatly impact biofilter removals. However, because LC-OCD is a relatively new technique used to study size fractions of NOM in drinking water treatment, more studies are needed that will provide additional comparative data.

Biofilter removal results show that temperature did not significantly affect the removal of humic substances, building blocks or LMW neutrals through the biofilters. However, temperature did affect biofilter removal of DOC, biopolymers and LMW acid and humics. The average removal of biopolymers through the biofilters was 39% and 15% at T>10°C and T≤10°C respectively, and the average removal of LMW acids and humics was 36% and 24% at T>10°C and T≤10°C respectively (Table 5.1). Rahman (2013) has previously reported that at temperatures between 10°C and 24°C, the concentration of biopolymers removed through biofilters was positively correlated with the biopolymer concentration in the feed, and that the percent removal of biopolymers through pilot-scale biofilters was essentially constant. Similar to the results in the present study, Peldszus *et al.* (2012) observed lower biopolymer concentrations in Grand River water at cold temperatures, which were accompanied by lower removal rates through direct biofiltration with no pre-treatment. In addition, since the biopolymers, and LMW acids and humics may be more biodegradable, reduced removal of these fractions at cold temperatures may be due to reduced microbial biodegradation within biofilters at

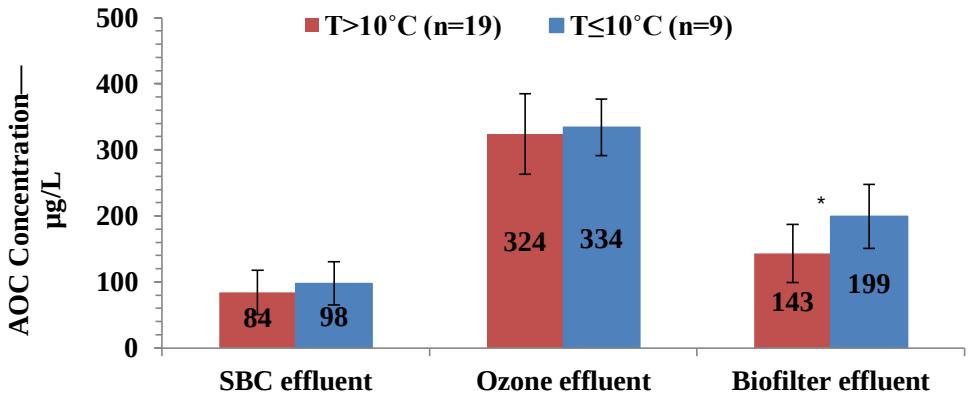
T≤10°C. The removal of other NOM fractions through the biofilters may not have been impacted by temperature as they are not as easily biodegradable. The reduced biopolymer removal observed through the biofilters at cold temperatures may be of concern to water treatment plants utilizing biofiltration as a pre-treatment to membranes, as biopolymers have been shown to be a major constituent of low-pressure membrane fouling (Hallé *et al.*, 2009).

During the present study, the UVA_{254} was reduced through the biofilters by approximately 0.003 cm^{-1} (n=31), representing a 5% reduction. The reduction in UVA_{254} by the biofilters was similar to the reduction in humic substances as quantified by LC-OCD of 6%. The similarity in the reduction in UVA_{254} and humic substances points to the removal of these compounds through biofiltration and not to a change in composition or aromaticity. Just like for humic substances, there was no change in UVA_{254} between the two temperature ranges. The use in the present study of a novel NOM characterization technique, LC-OCD, has provided valuable information on the removal of important NOM fractions in drinking water treatment processes. The constant removal of humic substances through ozone-biofiltration observed at different seasonal temperatures is of value due to the relationship that has been reported between humic substances and DBP formation (Wassink *et al.*, 2011).

5.4.2 Assimilable organic carbon (AOC)

The mean AOC concentration was 88 µg AOC/L (n=28) in the SBC effluent, and there was no statistically significant difference between the SBC effluent AOC concentrations through the year (Figure 5.3). Following ozone, the mean AOC concentration increased more than three-fold, to 327 µg AOC/L on average, with an essentially constant ozone effluent AOC concentration through the year. The increase in AOC observed at the HWTP after ozone was expected and is in line with what has been reported by others. For example, Lehtola *et al.* (2001) reported an average increase in AOC concentration of 157% following ozonation at six full-scale plants (residual O_3 dose ranged from 1.0 to 1.98 mg O_3/L). Escobar & Randall (2001) also reported AOC increases in the plant effluent from 112% to more than 200% after ozonation was implemented. Hammes *et al.* (2006) reported about a three-fold increase in AOC concentration following ozone at a full-scale surface water treatment plant. Variations in reported AOC values are expected, and Hammes and Egli (2005) have pointed to the use of different AOC methods as one source of this variation. Another source of variation is the applied/transferred ozone dose. The lack of relationship between temperature and concentration of AOC produced after ozone has also been previously reported by Vahala *et al.* (1998a).

Following ozone, the biofilters achieved a statistically significant AOC removal of 56% (n=19) at T>10°C, with a mean biofilter effluent AOC concentration of 143 µg AOC/L , while at T≤10°C the biofilters achieved a mean biofilter effluent AOC concentration of 199 µg AOC/L, resulting in 40% AOC removal (n=9) (Figure 5.3). The difference in biofilter effluent AOC concentrations observed at T>10°C and T≤10°C were statistically significant. The removal of AOC through the HWTP biofilters was similar to other results that have previously been reported in filters which use granular activated carbon (GAC) (e.g. Vasyukova *et al.*, 2013) and sand (e.g. Hammes *et al.*, 2006) as filter media. Similar removals have also been reported by Wang *et al.* (1995) who evaluated the efficiency of an anthracite-sand pilot biofilter in treating Ohio River water and observed 39% AOC-NOX removal at steady state. Additionally, Wert *et al.* (2008) reported up to 60% AOC removal through pilot-scale anthracite/sand biofilters of pre-ozonated lake water. The 38 minute EBCT in the present study may also contribute to the considerable AOC removal, as a previous study has shown that contact time can impact AOC removal, with somewhat longer contact times required to remove AOC (Huck *et al.*, 2000).



Error bars represent ± standard deviation.
*****—statistically significant difference in AOC concentration (p<0.05) between T>10°C and T≤10°C.

Figure 5.3: Mean AOC concentrations through the HWTP at water temperatures (T) >10˚C (n=19) and T≤10˚C (n=9)

Although Melin *et al.* (2002) and Persson *et al.* (2006) have found no relationship between readily biodegradable carbon removal by biofilters and temperature, a number of other studies have found that a temperature effect does exist. For example, Moll *et al.* (1999) reported that AOC removal through pilot-scale sand biofilters was reduced by 23% when operated at 5°C compared to 20°C and 35°C. In addition, Huck *et al.* (2000) reported reduced oxalate removal, used as a representative easily biodegradable compound, at temperatures between 1 and 3°C, compared to temperatures between 21

and 25°C, in anthracite/sand biofilters operated without ozone pre-treatment. The reduction in AOC removal observed at cold temperature through the biofilters may be due to the reduced microbial biodegradation, which, as mentioned previously, may have also impacted biopolymer and LMW acids and humics removal.

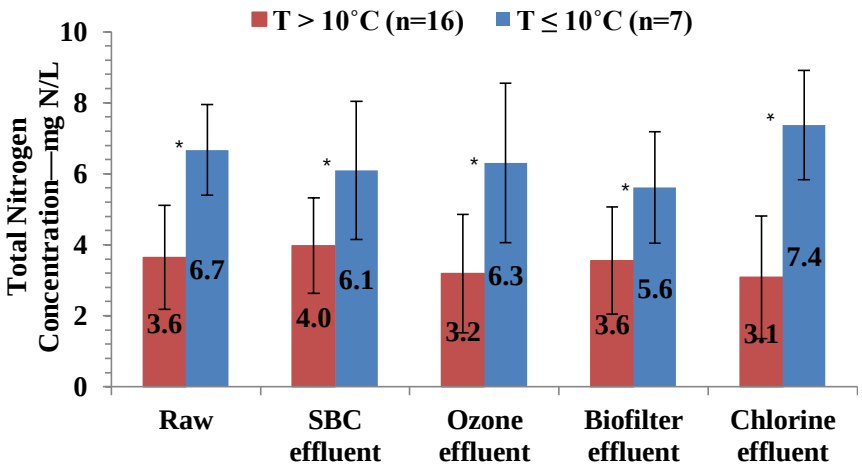
The changes in AOC and LC-OCD quantified NOM fractions observed through ozone-biofiltration in the present study were investigated further to determine if there was a relationship between these two NOM characterisation methods. Results demonstrate that ozone led to an increase of 0.24 mg AOC/L (280% increase) and an increase of 0.05 mg C/L of LMW acids and humics (40% increase). Through biofiltration, an average removal of 0.17 mg AOC-C/L (52% reduction) and 0.05 mg C/L LMW acids and humics (31% reduction) was observed. Although the concentration of AOC and LMW acids and humics followed a similar trend in that they both increase considerably after ozone, and exhibit temperature dependent removal through the biofilters, there was essentially no relationship between the amount of carbon removed as measured by AOC compared with NOM fractions quantified by LC-OCD (biofilter AOC removal and LMW acid and humics removal; Pearson's R = 0.05). These results point to the importance of performing AOC analyses, as a complimentary method to LC-OCD, to gain a greater understanding of overall NOM composition.

5.4.3 Nitrogen

There was no significant change in total nitrogen concentration through the HWPT (Figure 5.4).Total nitrogen concentrations were significantly higher in both the raw water and through the plant at colder temperatures (T≤10°C), with an average overall concentration of 6.7 mg N/L (n=7), compared to warmer temperatures (T>10°C) with an average overall concentration of 3.6 mg N/L (n=16) (Figure 5.4). A high amount of variability was observed in the total nitrogen concentrations as can be seen by the wide standard deviation at each sample location. Similar to the total nitrogen concentration, the concentration of nitrate was also higher at T≤10°C (Figure 5.5). The raw water nitrate concentrations reported in the present study are within the ranges reported by another recent study performed on the Grand River (Van Dyke *et al.*, 2010). In the raw water, nitrate-nitrogen made up on average 70% of the total nitrogen concentration. The increased concentration of nitrogen compounds observed in the Grand River water at cold temperatures may be due to increased concentrations in runoff in the fall and winter. With reduced plant growth at cold temperatures, the nitrogen containing compounds which would usually be taken up by plants are washed into the river. Others have reported that rivers within watersheds with significant agricultural activity, such as the Grand River watershed, can show dramatic

seasonal organic and inorganic nitrogen loading patterns, with higher nitrogen concentrations occurring during periods of high flow (Phipps & Crumpton, 1994). Additionally, in a study of the Grand River watershed, nitrate levels in some parts of the watershed were highest during December and January due to the groundwater that contributes to the flow (Cooke, 2006).

There was no nitrate removal by the treatment plant as was expected because an aerobic environment was maintained, and instead results showed a statistically significant increase in nitrate concentration through ozonation, biofiltration and chlorination at the plant. The nitrate concentration of the finished water was 4.5 mg NO_3-N/L on average, and remained well below the Ontario Drinking Water Quality Standard nitrate limit of 10 mg NO_3-N/L (Safe Drinking Water Act, 2002). Simon *et al.* (2013) also reported that biofiltration had no effect on nitrate removal and observed an increase in nitrate following expanded clay biofiltration of seawater. The authors point to the biological oxidation of ammonium and nitrite by nitrification as the cause for this increase (Simon *et al.*, 2013).

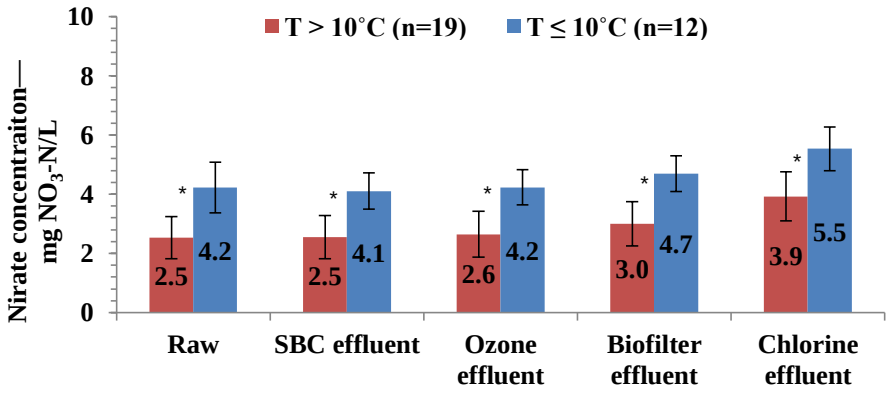


Error bars represent ±standard deviation.
*****—statistically significant difference in total nitrogen concentration (p<0.05) between T>10°C and T≤10°C

Figure 5.4: Mean total nitrogen concentrations through the HWTP at water temperatures (T) >10˚C (n=16) and T≤10˚C (n=7)

At the HWTP, the concentration of ammonia in the raw water was less than 0.58 mg/L, but varied greatly with temperature, as was expected, with low ammonia concentrations at warm temperatures and higher ammonia concentrations at cold temperatures (Figure 5.6). This is likely due to the increased volatilization of ammonia at elevated temperatures, and may also be due to the increased biological activity in the river at warm temperatures which may lead to increased conversion of ammonia to nitrate. This hypothesis is supported by the observation that when the river is ice-covered, ammonia

levels increase (Cheyne, 2008). The seasonal variation in ammonia in the raw water may also be due to the higher wastewater effluent ammonia discharge concentrations from upstream wastewater treatment plants during the winter (Earth Tech Canada Inc., 2007). As with the other nitrogen containing compounds, no removal of ammonia occurred through ozonation and biofiltration at the HWTP. The raw water ammonia concentrations reported in the present study are also within the ranges reported by another study performed on the Grand River (Van Dyke *et al.*, 2010). Previous studies have reported substantial ammonia removal through drinking water biofilters, although considerable temperature effects have been reported (Andersson *et al.*, 2001). In the present study, the lack of observed ammonia removal through the biofilters may be due to the limited growth of ammonia-oxidizing biomass, which may be limited due to the low raw water ammonia concentrations (Uhl & Gimbel, 2000), and due to the fact that the pre-ozonated biofilters had only been in operation for a few months when this study began. Furthermore, at cold temperatures, the rate of biological oxidation of ammonia to nitrite and subsequently to nitrate is reduced by approximately 50% (at 12°C) (Andersson *et al.*, 2001), which may further contribute to the lack of ammonia removal.



Error bars represent ±standard deviation.
*****—statistically significant difference in nitrate concentration (p<0.05) between T>10°C and T≤10°C

Figure 5.5: Mean nitrate concentrations through the HWTP at water temperatures (T) >10˚C (n=19) and T≤10˚C (n=12)

Although organic nitrogen was not specifically measured in the study, historical data from the Grand River in Brantford show that the average concentration of organic nitrogen, determined by subtracting ammonia and ammonium from the total Kjeldahl nitrogen, was 0.55 mg N/L (n=7) (DWSP, 2009). Compared to the total nitrogen compounds quantified in the present study, it is speculated that the concentration of dissolved organic nitrogen would represent a small fraction (12%) of the total nitrogen

concentration. The organic nitrogen detector within the LC-OCD also provides some indication of the organic nitrogen content in the biopolymer and humic substance fractions within the present study. In the biopolymer fraction, nitrogen atoms would likely be present in proteins, whereas in the humics fraction high nitrogen content may suggest that the humic substances are derived from effluent organic matter (Huber *et al.*, 2011). The organic nitrogen concentration can be used to calculate the nitrogen to carbon (N:C) ratio of the biopolymer and humic substance fractions. Results show that on average, the N:C ratio in the raw water biopolymer fraction was 0.10, and increased through the HWTP to 0.18 (Figure 5.7). These results suggest that there is preferential removal of carbon compounds at the HWTP. These results are contrary to those reported by Vasyukova *et al.* (2013) who reported that the N:C ratio of the biopolymer fraction was initially 0.22 on average, and changed to 0.1 through a full-scale plant comprising coagulation, flocculation, sedimentation, rapid sand filtration, ozone, biofiltration, and chlorine disinfection treating organic rich surface water. The average N:C ratio of the humic substance fraction in the raw water at the HWTP was 0.05 and remained constant through the treatment plant (Figure 5.7). This result is similar to that reported by Vasyukova *et al.* (2013) who reported an average N:C ratio of 0.03 for humic substances, which also remained constant through full-scale water treatment.

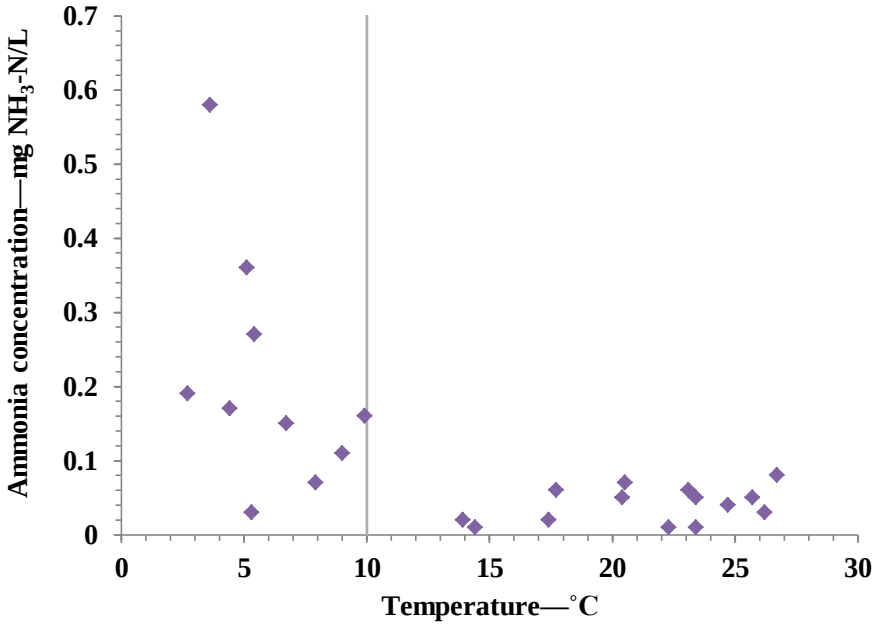
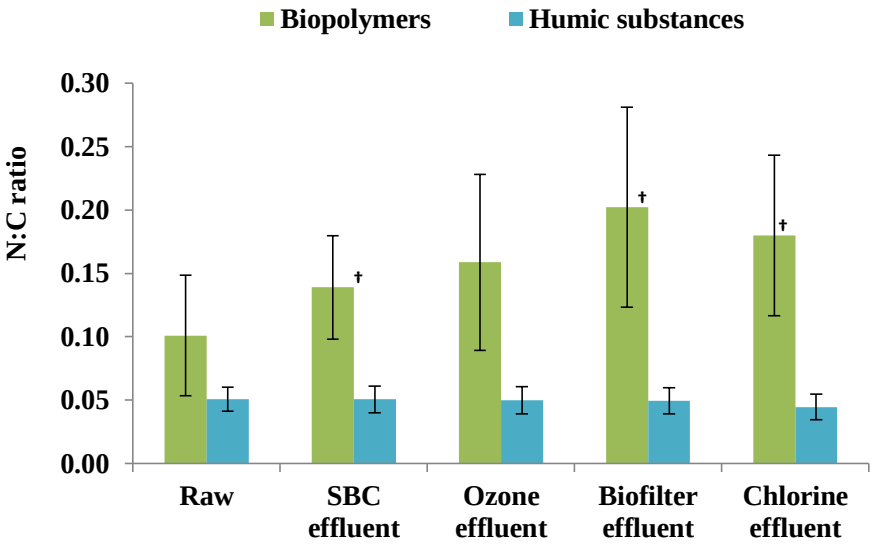


Figure 5.6: Raw water ammonia concentrations from May 2012 to July 2013

5.4.4 Biomass quantity and activity

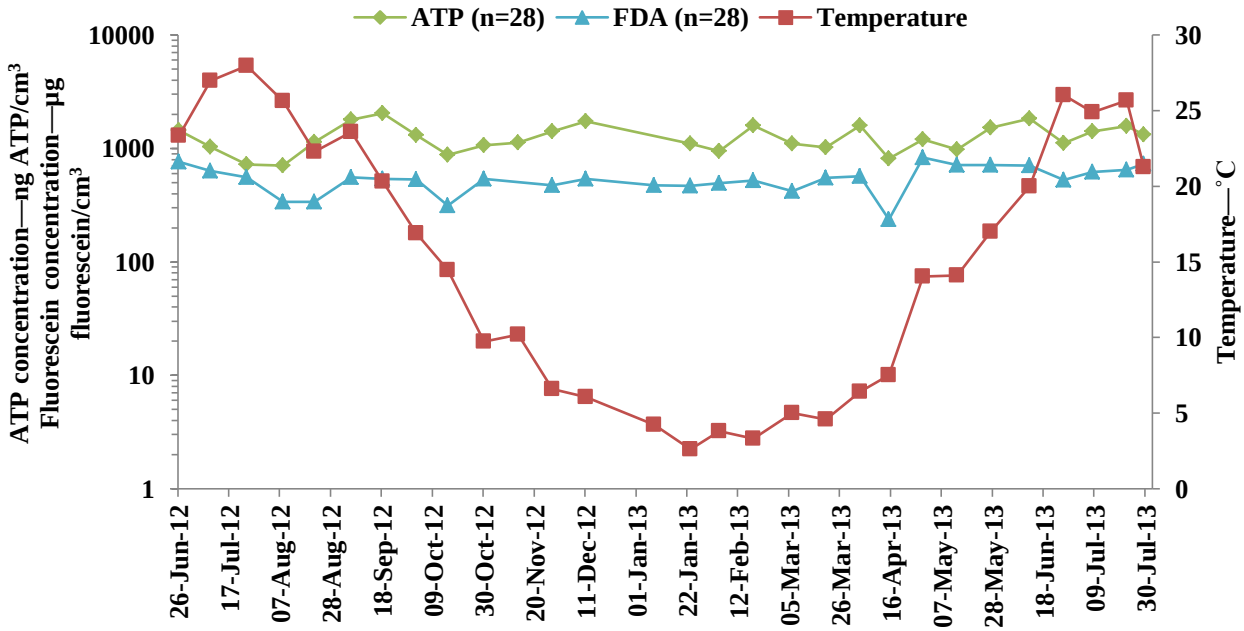
Investigation into the quantity and activity of biomass present within the biofilters was undertaken to determine if a relationship between biofilter performance and the quantity or activity of biomass existed. ATP was used as an indicator of the quantity of viable biomass, as it is the primary energy carrier in all types of living cells and is used for cell synthesis and maintenance (Rittmann & McCarty, 2001). Biomass activity was quantified by measuring FDA hydrolysis to fluorescein (Clark *et al.*, 2001). The amount of fluorescein produced is proportional to the amount of active enzymes within the biomass, providing a good indication of biomass activity (Leszczyñska and Oleszkiewic, 1996). Although the biofilters' ability to remove certain compounds, such as DOC, biopolymers, LMW acids and humics, and AOC were diminished at T≤10°C, the quantity and activity of the biomass remained essentially constant through the year (Figure 5.8). The average quantity of biomass measured just below the surface of the biofilter using the ATP method was 1,268 ng ATP/cm^3, and the average activity of the biomass using the FDA method was 548 µg fluorescein/cm^3. Both ATP and FDA hydrolysis followed similar trends, although very little variability was observed in both the ATP and FDA results, essentially no correlation was observed (Pearson's R=0.4). Compared to the ATP results published by others (e.g. Magic-Knezev & van der Kooij, 2004), the concentration of ATP reported suggests that the biofilters were acclimated and contained a considerable amount of biomass.



Error bars represent ± standard deviation
†—statistically significant (p<0.05) change from previous treatment step.

Figure 5.7: N:C ratio for biopolymers and humic substances through the HWTP (n=23)

Although bacterial growth is known to be impacted by temperature (Rittmann & McCarty, 2001), other studies have also shown that changes in temperature do not result in changes in ATP concentration at the surface of biofilters. For example, Rahman (2013) observed stable ATP concentrations at the surface of pilot-scale anthracite/sand biofilters operated with raw water temperatures between 10 and 24°C. Evans *et al.* (2013a) also observed that ATP concentrations did not vary considerably over time, even though measurable changes in temperature were observed, and that ATP and hydrolase enzyme activity, determined using the BactiQuant test kit (Mycometer, Tampa, Florida), correlated well. Based on results reported in the present study, the quantity and activity of biomass, as determined by ATP and FDA, do not provide a good indication of biofilter performance, as no change in ATP was observed with temperature, while considerable changes in biofilter performance were observed. Similar results have also previously been reported by Huck *et al.* (2000) in which essentially no relationship was observed between biomass (measured using the phospholipid method) and biofilter BOM removal. This discrepancy may be due to the fact that although bacterial metabolism is reduced at cold temperatures, overall bacterial cells numbers are not affected. In addition, the enzymatic activity measured by the FDA method may not be correlated with organic matter metabolism and may not be as greatly impacted by temperature.



ATP—Adenosine triphosphate, FDA-Fluorescein diacetate.

Figure 5.8: Biomass quantity (ATP) and activity (FDA) from May 2012 to July 2013

5.5 Conclusions

The removal of organic matter through drinking water treatment processes improves the aesthetic quality and biostability of water, and can lead to the removal of precursors to potentially harmful disinfection by-products. At the HWTP, the performance of the ozone-biofiltration process was investigated to determine the effect of seasonal changes in raw water temperature and quality on carbon and nitrogen compound removal.

The following conclusions can be made:

- Statistically significant changes in raw water biopolymer, total nitrogen, nitrate, and ammonia concentrations were associated with seasonal water temperature changes. Biopolymer concentrations were higher at T>10°C, while nitrogen compound concentrations, total nitrogen, nitrate, and ammonia were higher at T≤10°C
- The ozone-biofiltration process achieved statistically significant overall removal of all NOM fractions with 15% DOC removal, 35% biopolymer removal, 10% humic substance removal, 12% building block removal, 3% LMW acid and humic removal, and 17% LMW neutral removal
- DOC, biopolymer, and LMW acids and humics removal through biofiltration was reduced at T≤10°C (statistically significant)
- The biofilters achieved considerable assimilable organic carbon removal through the year (52% overall), although removal was lower at T≤10°C (40%) compared with 56% at T>10°C
- No removal of total nitrogen, nitrate, or ammonia through ozone-biofiltration was observed at the HWTP, although no nitrate removal was expected.
- The biomass quantity and activity within the biofilters remained constant through the year thus demonstrating the lack of relationship between organic carbon removal and biomass, as quantified by ATP and FDA hydrolysis.

Chapter 6
Nutrient Availability in Drinking Water Treatment Biofilters

6.1 Overview

Biofilters used in drinking water treatment contain media on which biomass is allowed to grow. This biomass forms a complex environment, which includes cell attachment, detachment, growth, and decay. The growth of biomass, however, is dependent on the concentration of macronutrients available in the biofilter feed water, which can be considerably affected by source water quality, upstream treatment processes, and biomass decay. The present study investigated the impact of carbon, nitrogen and phosphorus ratios on biofilter performance in a full-scale drinking water treatment plant. In addition, the effect of biofilter pre-treatment processes, such as sand-ballasted clarification (SBC) and ozone, on nutrient availability was determined. Results demonstrated that SBC substantially reduced the phosphorus concentration, leading to carbon:nitrogen:phosphorus (CNP) ratios in the biofilter feed of less than 100:10:1. Although possible nutrient limitations in the biofilter feed were calculated, the biofilters demonstrated substantial assimilable organic carbon removal, and maintained a high level of biomass quantity and activity. These results suggest that good biofilter performance can be achieved in waters that exhibit CNP ratios thought to be less optimal.

6.2 Introduction

Bacterial biofilms are produced when cells attach to solid surfaces and undergo colonization, in which they grow and produce extracellular polymeric substances (EPS), which act as a scaffold providing support to the biofilm (Lauderdale *et al.*, 2012; Madigan & Martinko, 2006; Watnick & Kolter, 2000). Ongoing biofilm development is impacted by cell growth and EPS formation, attachment, detachment and decay (Madigan & Martinko, 2006; Hozalski & Bouwer, 2001). Bacteria form biofilms because it creates an environment where fresh substrates pass by them at all times, and biofilms also provide protection from harmful compounds such as chlorine (Madigan & Martinko, 2006; Rittmann & McCarty, 2001). Cells within the biofilm obtain nutrients through diffusion of molecules from the bulk fluid into the biofilm (Rittmann & McCarty, 2001), and may also obtain nutrients from the decay of neighbouring microorganisms. Many nutrients are essential for bacterial cell growth and for use as an energy source, although the concentration required fluctuates for each nutrient and is dependent on the bacterial cell type (Madigan & Martinko, 2006). Macronutrients are required in larger amounts by bacterial cells and include carbon, nitrogen, and phosphorus (Madigan & Martinko, 2006).

Micronutrients are required in lesser amounts and include sulfur, magnesium, sodium, potassium, chloride and metals such as iron, manganese, boron, zinc and copper (Madigan & Martinko, 2006), and are typically not limiting in natural waters. Some bacteria may also require additional growth factors that they cannot synthesize including vitamins and amino acids, although the concentration and type required fluctuates considerably between bacterial types (Madigan & Martinko, 2006).

As biofilms occur naturally in the environment they have been found in engineering processes such as drinking water treatment plants (DWTPs) (Rittmann & McCarty, 2001). Biofilms have been shown to be present along the walls of water distribution tanks and piping, and can form around media in filters, creating biological filters (biofilters) (e.g. Huck & Sozański, 2008; Ridgway & Olson, 1981). Within distribution systems, biofilms can have negative effects, contributing to corrosion, decreasing the aesthetic quality of drinking water, and increasing the disinfectant demand (Chu *et al.*, 2005; Sathasivan *et al.*, 1997). However, biofilms within filters contribute to the production of biologically stable drinking water by removing biodegradable organic matter (BOM) (e.g. Urfer *et al.*, 1997). Due to the beneficial effects biofilms have within filters, their growth is not discouraged and in some instances can be enhanced. For example, ozone can be located prior to biofilters to oxidize natural organic matter into more biodegradable forms. Studies evaluating the nutrients that control regrowth of bacteria within distribution systems have found that carbon is typically the growth limiting nutrient in drinking water treatment plants (LeChevallier *et al.*, 1991), although phosphorus limitations have also been reported following ozonation in water treatment plants (Lehtola *et al.*, 2001) and throughout distribution systems (Miettinen *et al.*, 1997).

The major nutrients required for cell growth are often expressed by the carbon:nitrogen:phosphorus (CNP) molar ratio. Molecular nutrient ratios were originally developed in the early 1900s and are based on the nutrient composition of phytoplankton, of 100:15.4:1.88 parts by weight, which were found to be similar to the nutrient composition of the ocean, of 100:16.7:1.85 parts by weight (Redfield, 1934). For microbial growth in drinking water systems, a CNP ratio of 100:10:1 has typically been assumed to be required for cell synthesis (e.g. LeChevallier *et al.*, 1991). However, the exact ratio can depend on a number of factors including microorganism type, environment, etc. (Vrede *et al.*, 2012). In engineering applications, the CNP ratio is often used in wastewater treatment to ensure sufficient nutrients are available for activated sludge processes and biomass synthesis (Tchobanoglous *et al.*, 2003). More recently, researchers have used the CNP ratio to determine and optimize nutrient availability in biofilters (e.g. Lauderdale *et al.*, 2012).

The goal of the present study was to assess nutrient availability in a full-scale DWTP which included biofilters. The concentrations of major nutrients were determined and their removal prior to biofiltration through water treatment processes was investigated. The relationship between nutrient availability, CNP ratio, and biofilter performance was investigated to identify potential nutrient limitations at the treatment plant.

6.3 Materials and Methods

6.3.1 Holmedale Water Treatment Plant

The present study was undertaken at the Holmedale Water Treatment Plant (HWTP) which is located in Brantford, Ontario and uses the Grand River as its source. The treatment process consists of sand-ballasted clarification (SBC), ozonation, biofiltration, ultraviolet light, and chlorine for primary disinfection, followed by ammonia addition to form monochloramine for secondary disinfection. During the present study, the average polyaluminum chloride concentration added was 37 mg/L and the average ozone dose was 1 mg/L. The ozone process was operated to ensure no ozone residual reached the biofilters. The biofilters contained 1.4 m of anthracite over 0.6 meters of sand, and they were backwashed with non-chlorinated water. More information relating to the treatment processes employed at the HWPT can be found in Chapters 4 and 5.

6.3.2 Sample collection and analysis

Between April and July 2013, samples were collected from the raw water, SBC effluent, ozone effluent and biofilter effluent. They were analysed for the following parameters: dissolved organic carbon (DOC), assimilable organic carbon (AOC), ammonia, nitrate, total nitrogen (TN), orthophosphate, total dissolved phosphorus (TDP), and total phosphorus (TP). Sample collection was performed on 10 occasions. Further information relating to sample collection and the DOC, AOC and nitrogen compound analyses can be found in Chapter 5. DOC, AOC, ammonia, nitrate and TN were analysed at the laboratory at the University of Waterloo, while phosphorus containing compounds were analysed by ALS Environmental (Waterloo, Ontario). The detection limit for the TN method was 0.05 mg/L, for the nitrate method was 0.1 mg NO3—N/L, and for the ammonia method was 0.02 mg NH3-N/L. Phosphorus compounds were quantified according to Standard Method 4500-P B E (2012), with TDP samples filtered through a 0.45 μm filter prior to analysis. The detection limit for the TP, TDP and orthophosphate analyses was 0.0030 mg P/L.

Biofilter performance was determined by quantifying the amount of biomass using adenosine triphosphate (ATP), and biomass activity was measured using fluorescein diacetate (FDA) hydrolysis analysis. Specific method information for both the ATP and FDA hydrolysis methods can be found in Chapter 5.

6.3.3 Statistical analysis

For statistical analysis, samples with a result below the method detection level were given a value of half the detection limit (USEPA, 2000).

6.4 Results and Discussion

In the present study, nutrient concentrations through the HWTP were monitored to determine if they had an impact on biofilter performance and activity. Carbon, nitrogen and phosphorus concentrations, including both total and biologically available forms, were monitored. For carbon, DOC and AOC were quantified as DOC provides an indication of the total dissolved organic carbon concentration and AOC provides a measure of the readily biodegradable organic carbon. Three forms of nitrogen were quantified, including TN and two forms of biologically available nitrogen, ammonia and nitrate. Although most bacteria use ammonia as a source of nitrogen, many types of bacteria can also use nitrate (Madigan & Martinko, 2006). Phosphorus was determined by measuring the TP concentration and the TDP concentration, to identify the fraction of phosphorus in the particulate form. Additionally, a form of phosphorus that can be readily used by microorganisms, orthophosphate, was also determined.

A more detailed discussion of carbon and nitrogen in the raw water and their removal through the HWTP has previously been provided in Chapters 4 and 5. Therefore, this chapter will focus on phosphorus changes through the DWTP, and comparing CNP ratios to biofilter performance (AOC removal) and activity.

6.4.1 Raw water phosphorus concentration

Over the course of the study, raw water nutrient concentrations were monitored (Table 6.1). Phosphorus in water originates from many different sources, including detergents, fertilizers, manure, human waste, and decaying plants (Environment Canada, 2013), therefore its concentration in water can vary considerably based on upstream activities, including wastewater discharge. The average TP concentration in the raw water was 0.048 mg P/L, which is lower than the overall mean reported for the Grand River below Brantford of 0.1 mg/L by MacDougall & Ryan (2012). Results demonstrate that

TDP contributed to between 26% and 89% of the TP in the raw water, and was on average 0.025 mg P/L. The orthophosphate concentration in the raw water was 0.013 mg PO_4-P/L. Throughout this investigation, the average temperature in the Grand River was 18°C, with temperature fluctuations from a low of 5°C to a high of 26°C (n=10). The orthophosphate and TDP concentrations in the raw water did not appear to be related to raw water temperature (Figure 6.1).

Table 6.1: Carbon, nitrogen, phosphorus concentrations through the HWTP

Biofilter feed	n	Raw water mean (s.d.)	SBC effluent mean (s.d.)	Ozone effluent mean (s.d.)	Biofilter effluent mean (s.d.)
Carbon					
Assimilable Organic Carbon (AOC) (mg C/L)	10	-	0.10 (0.04)	0.34 (0.06)	0.16 (0.05)
Dissolved Organic Carbon (DOC) (mg C/L)	10	6.08 (0.47)	4.02 (0.33)	3.88 (0.30)	3.36 (0.22)
Nitrogen					
Ammonia (mg NH_3-N/L)	8	0.05 (0.02)	0.04 (0.02)	0.06 (0.03)	0.04 (0.03)
Nitrate (mg NO_3^-N/L)	10	3.02 (0.86)	3.13 (0.88)	3.16 (1.02)	3.64 (0.81)
Total Nitrogen (mg N/L)	6	3.27 (0.56)	3.53 (1.18)	3.92 (1.11)	3.83 (1.24)
Phosphorus					
Orthophosphate (mg PO_4-P/L)	10	0.013 (0.011)	LDL*	LDL*	LDL*
Total Dissolved Phosphorus (mg P/L)	10	0.025 (0.010)	0.006 (0.004)	0.004 (0.002)	0.009 (0.013)
Total Phosphorus (mg P/L)	10	0.048 (0.018)	0.010 (0.004)	0.016 (0.013)	0.005 (0.003)

*All measurements were below the method detection limit of 0.0030 mg PO_4-P /L.
LDL—lower than detection limit, n—number of sample events, s.d.—standard deviation.

6.4.2 Effect of biofilter pre-treatment on phosphorus availability

Water treatment processes that precede biofilters can have a substantial impact on biofilter nutrient availability. The impact of SBC and ozone on carbon and nitrogen in Chapters 4 and 5 showed that SBC contributed to significant DOC removal (34%, n=10) while ozone contributed on average to 13%

DOC removal (n=10). Both the SBC and ozone did not significantly impact ammonia concentration, although an increase in nitrate and total nitrogen were observed following ozone.

The SBC process was shown to remove considerable concentrations of phosphorus-containing compounds (Table 6.1). Orthophosphate was removed to below the method detection limit (0.0030 mg P/L) on all but one occasion. The SBC process also reduced both the TDP and TP concentrations by approximately 80%, down to 0.006 mg P/L and 0.010 mg P/L, respectively (Table 6.1). Considerable removal of phosphorus through SBC processes has been previously reported by Plum *et al.* (1998). Similarly the removal of phosphorus through coagulation has also previously been reported due to the ability of coagulants, such as polymers and alum, to precipitate phosphate (Tchobanoglous *et al.*, 2003). The ozone process had little effect on the TDP and TP concentration, confirming what has been reported for TP by Lehtola *et al.* (2001). However, others have reported that ozone can increase the amount of microbially available phosphorus in water, even when pretreated by coagulation (Lehtola *et al.*, 2001).

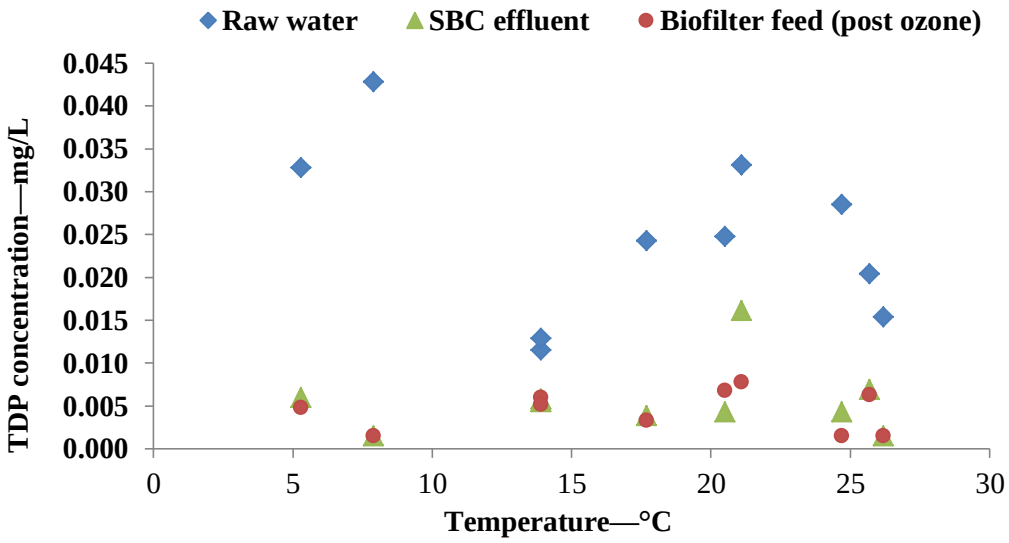


Figure 6.1: Total dissolved phosphorus (TDP) concentration through the HWTP

6.4.3 Biofilter nutrient availability

During this 4 month investigation, the average concentrations of carbon-containing compounds in the biofilter feed were 3.88 mg DOC /L and 0.34 mg AOC /L (Table 6.1). The ammonia concentration in the biofilter feed was 0.06 mg NH4-N/L, the average concentration of nitrate was 3.16 mg NO_3-N/L and the average TN concentration was 3.92 mg N/L (Table 6.1). The orthophosphate concentration was

below the method detection limit, the TDP concentration was 0.004 mg/L, and the TP concentration was 0.016 mg/L. Some removal of TP was observed through the biofilters, while no substantial removal of TDP was observed. These results suggest that there was no significant uptake of TDP within the biofilters or more likely that it was recycled within the biofilm.

CNP ratios were calculated for each sample collection day, using biologically available forms of carbon, nitrogen and phosphorus present within the biofilter feed including AOC, ammonia and TDP. AOC concentration was used for this calculation as it represents the most readily biodegradable organic carbon fraction which provides energy and carbon to bacteria (Standard Methods, 2012). Ammonia was used as the nitrogen source in the CNP ratio as most bacteria are capable of utilizing ammonia as their sole source of nitrogen. However, it is expected that this value would be an underestimation, since many microorganisms are also capable of using nitrate as a nitrogen source (Madigan & Martinko, 2006). Due to the fact that the orthophosphate concentration was below the method detection limit in the biofilter feed throughout the present study, TDP was used as an overly conservative phosphorus source to calculate CNP ratios. Although orthophosphate is traditionally considered to be the phosphorus form most readily available to bacteria, it is suspected that at low orthophosphate concentrations bacteria would be able to utilize phosphorus in other dissolved forms. This is because bacteria can hydrolyse dissolved organic phosphorus, including specifically polyphosphates, into orthophosphate (Wetzel, 1975; Tchobanoglous *et al.*, 2003). Throughout the present study, the CNP ratio was compared to the benchmark nutrient ratio of 100:10:1, however, it is important to remember that the CNP ratio may not always be constant (Cotner *et al.*, 2010). Previous studies have demonstrated that freshwater bacteria can be extremely phosphorus limited, with a study of the biomass composition in a US lake reporting a CNP ratio of 259:69:1, or 100:27:0.4 (Cotner *et al.*, 2010).

During this investigation, the carbon to nitrogen (CN) ratio was greater than 100:10 on all but two occasions and no relationship was observed between CN ratio and raw water temperature (Figure 6.2). Due to the presence of a considerable amount of nitrate in the biofilter feed (3.16 mg/L compared with 0.06 mg/L ammonia), it is speculated that the microorganisms would also be capable of utilizing nitrate as a nitrogen source in the event of an ammonia limitation. Therefore, it is expected that nitrogen would not be limiting in the biofilter feed. Determination of the carbon to phosphorus (CP) ratio at the HWTP using AOC and TDP shows that the phosphorus component of the CP ratio varied between 0.15 and 1.01 with a carbon molar ratio of 100 (Figure 6.2). The CP ratio was not affected by raw water

temperature (Figure 6.2). When compared to the reference CNP ratio of 100:10:1, on all but one sample collection day the ratio would suggest possible phosphorus limitations.

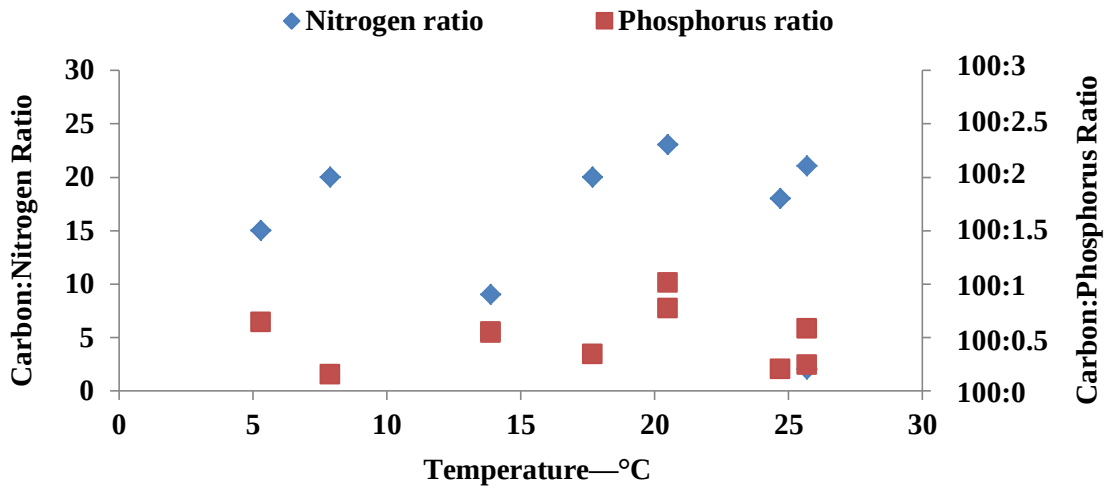


Figure 6.2: Nutrient ratio in the biofilter feed as a function of temperature

CN and CP ratios were then compared with biofilter performance, to evaluate the relationship between the nutrient ratios, and AOC removal through the biofilters. Results show that there was no relationship between CN or CP ratio, and AOC removal (Figure 6.3 and 6.4). These results suggest that biofilter performance, in terms of AOC removal, was not substantially impacted by a CP ratio that was considered less than optimal. It may be that the cells within the biomass have access to nutrients which are not quantified in the biofilter feed. Within biofilms, when cells die and subsequently lyse, their nutrients are released and made available to surrounding cells (Bayles, 2007). As cells within naturally developing biofilms are typically composed of 2 to 3% phosphorus by weight (Rittmann & McCarty, 2001), these recycled nutrients may be present in sufficient concentrations to avoid phosphorus limitations within biofilters. In addition, it is possible that the concentration of recycled nutrients, both nitrogen and phosphorus, could be lower immediately after backwash. However, Huck *et al.* (2000) found that backwash had no measureable effect on biomass levels at the surface of biofilters, as measured by a phospholipid method.

The relationship between the CN or CP ratio and the biomass quantity and activity was also investigated. The quantity of viable biomass within the biofilters was determined using adenosine triphosphate (ATP), and the activity of the biomass using fluorescein diacetate (FDA) hydrolysis. As a primary energy carrier in cells, ATP can provide an indication of the amount of viable cells within the

biomass, as has been previously discussed in Chapter 3 (Rittmann & McCarty, 2001). FDA hydrolysis analysis provides an indication of the activity of the biomass, as fluorescein hydrolysis

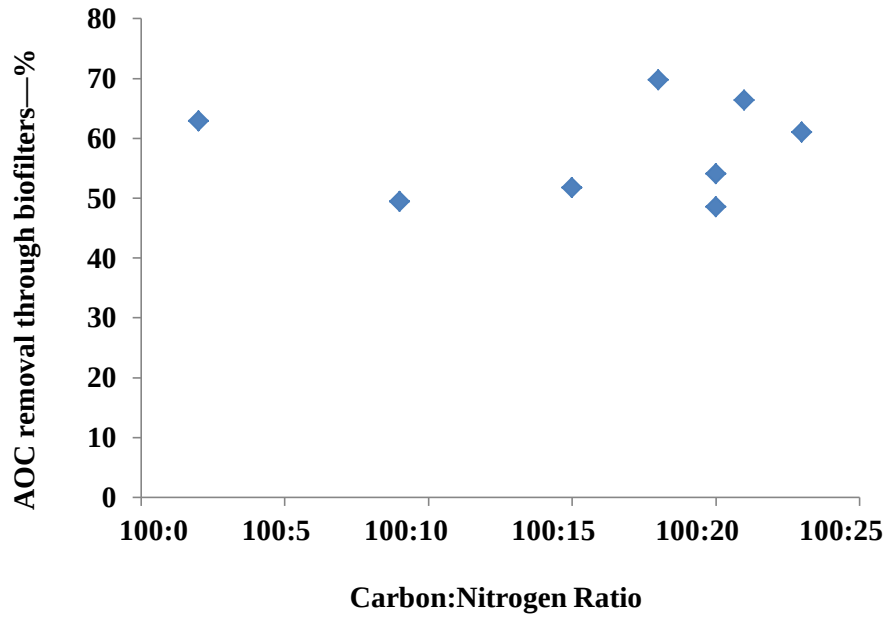


Figure 6.3: AOC removal by the biofilter vs. carbon:nitrogen ratio of the biofilter feed water

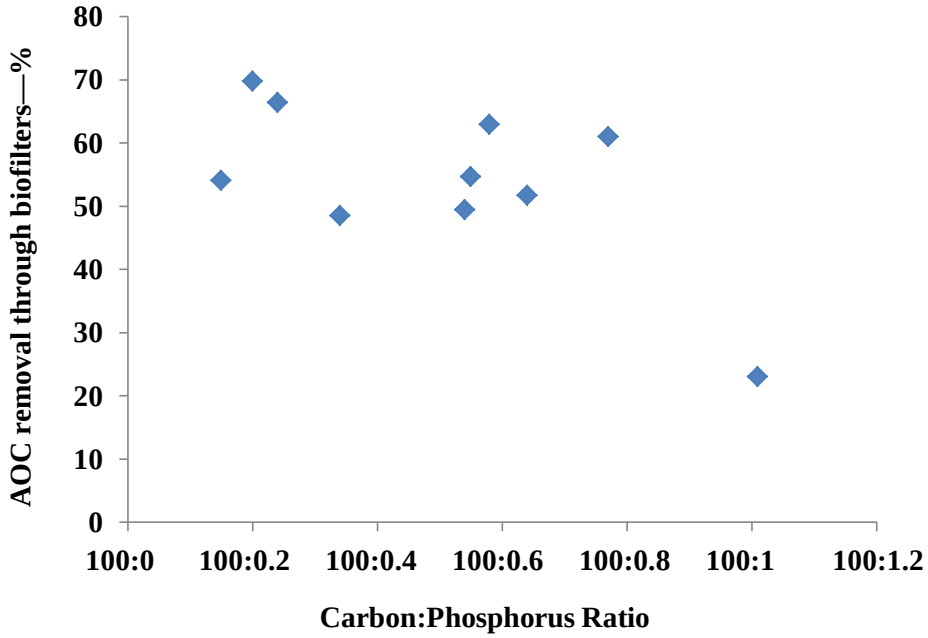


Figure 6.4: AOC removal by the biofilter vs. carbon:phosphorus ratio of the biofilter feed water

occurs in the presence of active microbial enzymes (Clark *et al.*, 2001; Leszczyñska and Oleszkiewic, 1996). The results from the present study unexpectedly illustrate that, similar to AOC removal, there is no apparent relationship between CN or CP ratio in the biofilter feed water and ATP or FDA hydrolysis in the biomass (Figure 6.5 and 6.6). Results indicate that although there was an apparent phosphorus limitation in the feed water, the quantity and activity of biomass were high. The biomass quantity determined by ATP analysis in the present study was higher than the ATP concentration reported in a pilot-scale anthracite/sand biofilter treating Grand River water upstream of the City of Brantford (Hallé, 2009). In that study, the biofilter was operated without pre-treatment (Hallé, 2009) except for potential chemically unassisted settling in the raw water storage basins, suggesting that the nutrient content in the biofilter feed would be virtually identical to the nutrient content in the Grand River.

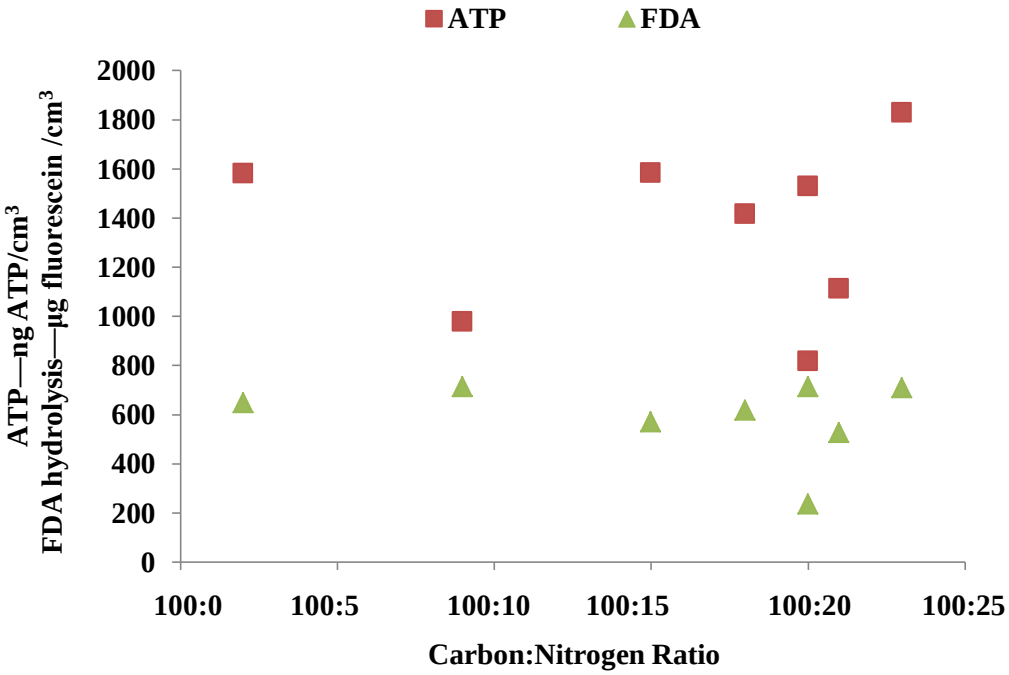


Figure 6.5: Impact of carbon:nitrogen ratio of the biofilter feed water on biomass quantity (ATP) and activity (FDA)

The lack of a relationship between CN and CP ratio and biofilter performance and biomass quantity and activity suggests that nutrients were present in sufficient concentrations. These results also demonstrate that even in the presence of very low orthophosphate concentrations, less than 0.0030

mg/L, significant AOC removal and biomass can be present within biofilters, suggesting that bacteria may be capable of utilizing other forms of phosphorus for growth. This should be considered when calculating CNP ratios, and that all forms of organic and inorganic nutrients should be considered as potential contributors to microbial growth requirements.

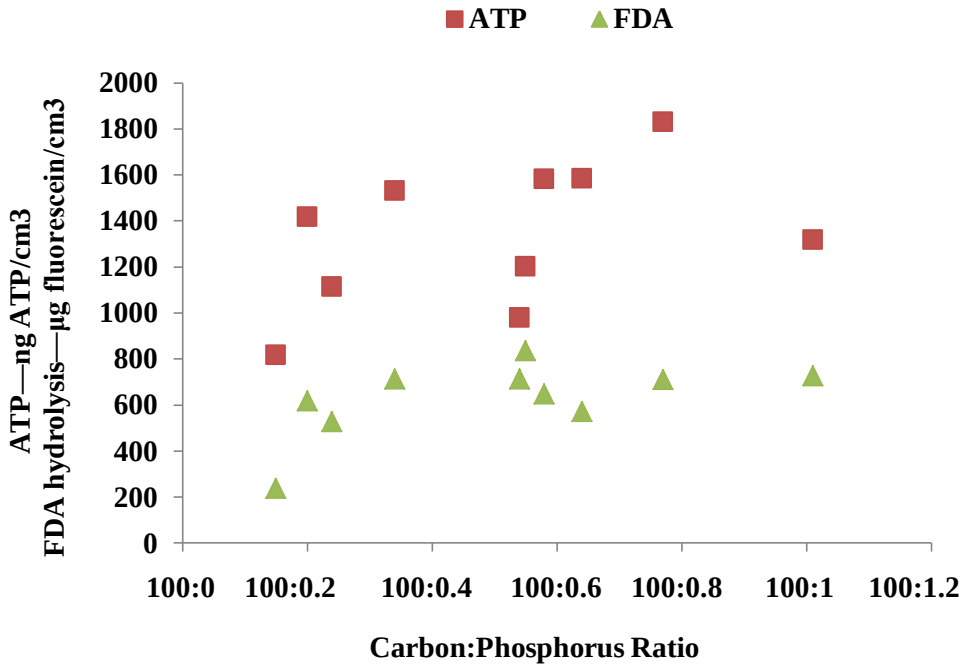


Figure 6.6: Impact of carbon:phosphorus ratio of the biofilter feed water on biomass quantity (ATP) and biomass activity (FDA)

In the present study, results demonstrate that variations in the CNP ratio did not substantially impact biofilter performance in terms of AOC removal or biomass quantity and activity. Although nutrient enhancement was not performed in the present study, other studies have evaluated the effects of nutrient dosing on biofilter performance. Similar to the results observed in the present study, Vahala *et al.* (1998b) demonstrated that very little difference was observed in AOC removal between biofilters with nutrient limitations and those with phosphorus addition, and also that nutrient addition did not lead to increased ATP of the attached biomass. Rahman (2013) also reported that in pilot-scale anthracite/sand biofilters treating river water with high humic concentration (over a temperature range of 10 to 24°C), phosphorus addition did not affect the biomass quantity (determined by ATP), the biomass activity (determined by FDA hydrolysis) or organics removal. However, others have shown that biofilter

performance can be improved with orthophosphate addition (Lauderdale *et al.*, 2012; Sang *et al.*, 2003; Yu *et al.*, 2003; Nishijima *et al.*, 1997). Lauderdale *et al.* (2012) dosed phosphoric acid to achieve a biofilter feed CP ratio of 100:2, and pilot-scale biofilters demonstrated increased DOC removal following nutrient enhancements, with 75% more DOC removed in the nutrient enhanced biofilter, compared to a control biofilter. Sang *et al.* (2003) and Yu *et al.* (2003) also showed that adding phosphorus to the influent of biofilters, with bio-ceramic media and GAC-sand, respectively, increased bacterial growth potential and chemical oxygen demand (permanganate consumption [CODMn]) removal. Although previous studies have demonstrated improved biofilter performance with nutrient enhancements, nutrient enhancement was not possible at the time of study at the HWTP. However, due to the low concentration of orthophosphate in the biofilter feed at the HWTP, the effect of nutrient enhancement at the HWTP may of interest.

6.5 Conclusions

The biomass within biofilters requires macronutrients, including carbon, nitrogen and phosphorus, in sufficient amounts for microorganisms to grow and for use as an energy source. Although nutrients at concentrations sufficient to support cell growth can typically be found in most surface water bodies, water treatment processes which precede biofilters may considerably reduce nutrient concentrations, leading to theoretical nutrient limitations. Results from the present study indicate that SBC had a statistically significant impact on phosphorus compound concentrations, leading to calculated nutrient limitations in the biofilter feed. However, the nutrient limitations determined according to the CNP ratio in the biofilter feed did not substantially impact biofilter performance, and biomass quantity and activity. Although previous studies have demonstrated that the addition of nutrients appears to at least temporarily enhance biofilter performance, results from the present study demonstrate that good biofilter performance and biomass quantity can be attained at CNP molar ratios of less than 100:10:1 in the biofilter feed. In fact, observations suggest that for this water type a CNP ratio of 100:10:0.1 may be sufficient for good biomass development in biofilters.

Chapter 7
Conclusions and Recommendations

The research presented in this thesis was performed to address the following goals: (1) the quantification of carbon and nitrogen removal through a full-scale municipal drinking water treatment plant (DWTP) employing sand-ballasted clarification (SBC), ozone, and biofiltration, and (2) investigation into full-scale biofilter performance and biomass activity/quantity. These goals were selected based on the importance of removing carbon and nitrogen compounds as they have been shown to act as precursors to the formation of DBPs. Additionally, confirming pilot-scale biofilter performance observations at full-scale provides valuable information for future design and upgrade of municipal DWTPs. The opportunity to perform this research at the HWTP in Brantford, Ontario, provided unique opportunities as the treatment plant includes less common processes, such as SBC, and unusual operational parameters, such as long biofilter empty bed contact times (EBCTs) (38 minutes). In addition, the significant variations in seasonal Grand River water quality and temperature (3-28°C) allowed for investigation into the seasonal performance of full-scale DWTP processes.

A review of the available literature was performed to determine what biomass quantity, as determined by ATP, is typical for active, acclimated drinking water treatment biofilters. This review identified that a benchmark of 10^2 to 10^3 ng ATP/cm^3 of biofilter media represents active, acclimated biofilters associated with anthracite or granular activated carbon (GAC). For biofilters with sand media, preliminary observations indicate that the ATP concentration is one order of magnitude less than in GAC and anthracite biofilters. Additionally, ATP concentrations at the surface of acclimated biofilters do not appear to be impacted by water source, temperature, EBCT, and media type (either anthracite or GAC). However, influent DOC, hydraulic loading rate, and pre-ozonation have a positive effect on the ATP concentration with pre-ozonation resulting in a two to three fold increase in ATP concentration at the surface of biofilters.

7.1 Summary of Conclusions

This 14-month study was performed to investigate raw water carbon and nitrogen concentrations and their removal through a full-scale municipal DWTP, utilizing traditional water quality parameters and a novel NOM characterization technique, liquid chromatography-organic carbon detection (LC-OCD). In addition, investigation into the performance of the full-scale biofilters and into the quantity and activity

of the biomass was included. This study also included investigation into the essential nutrients available for biomass growth within the biofilters.

Chapters 4 and 5 report on the investigation of carbon and nitrogen concentrations in the raw water, and removal through the various processes at the Holmedale Water Treatment Plant (HWTP), and are summarized below:

- Grand River concentrations of biopolymers, total nitrogen, nitrate, and ammonia varied substantially with seasonal temperature changes, with elevated concentrations of biopolymers at warm temperatures (greater than 10°C) and elevated concentrations of nitrogen-containing compounds at cold temperatures (less than or equal to 10°C).
- Throughout the year, sand ballasted clarification (SBC) achieved statistically significant TOC and NOM fraction removal without employing pH suppression. The average TOC removal at 30%, exceeded the USEPA's 25% required removal of TOC by enhanced coagulation for plants using conventional coagulation (25%) for this water type (average TOC = 6.31 mg/L; alkalinity = 187 mg/L). The removal of most NOM components through SBC was not significantly impacted by seasonal changes in raw water character and/or temperature.
- Ozone contributed to significant increases in biodegradable organic matter (BOM) as observed by increases in assimilable organic carbon (AOC), and low molecular weight (LMW) acids, and humics. In this respect, ozonation was virtually unaffected by seasonal changes.
- Biofiltration contributed to statistically significant removals of DOC, AOC, and all NOM fractions throughout the year. However, the performance of the biofilters, in terms of DOC, AOC, biopolymer, and LMW acid and humic removal at cold water temperatures was statistically significantly reduced.
- No removal of total nitrogen, nitrate, or ammonia was observed through SBC, ozone, or biofiltration under the conditions employed at the HWTP.

Based on material presented in Chapters 5 and 6, the following conclusions can be made about the biofilter biomass quantity and activity, and nutrient concentrations in drinking water treatment biofilters.

- The quantity of viable biomass present within the biofilters, determined by ATP, was quite substantial, and remained essentially constant throughout the year. The activity of the biomass, determined by fluorescein diacetate (FDA) hydrolysis, was also quite considerable, and also remained constant throughout the year despite variations in raw water temperature from 3 to 28°C.
- Biomass quantity and activity as assessed using ATP and FDA were not correlated with biofilter performance in terms of organic carbon removal efficiency.
- SBC, functioning as a biofilter pre-treatment process, contributed to statistically significant removals of orthophosphate, total dissolved phosphate (TDP), and total phosphate (TP).
- Although biofilter feed CNP ratio, determined using AOC, ammonia, and total dissolved phosphorus, appears to indicate a nutrient limitation, biofilter performance in terms of AOC removal, and biomass quantity and activity, were unaffected.

The present study provides unique and valuable insights into the full-scale performance of a municipal drinking water treatment plant. However, as the performance of unit processes is often impacted by influent organic matter concentrations, it is important to consider the order of treatment processes at the HWTP. As the first process at the HWTP, SBC contributed to substantial removal of organic matter and in particular phosphorus-containing compounds. Although the performance of ozonation and biofiltration at the plant was excellent, it might have been negatively or positively impacted by the upstream SBC process. For example, it is speculated that ozonation would lead to the production of greater concentrations of AOC if the influent organic matter concentration to the ozone process was increased (i.e. no SBC or changes in its operation). This could subsequently lead to increased biomass growth within the biofilters, and therefore increased organic matter removal. The trade-off between installing SBC prior to ozone/biofiltration and not including such as process for targeted NOM fraction removal would need to be assessed for distinct water types (and treatment goals). Therefore, it is important to consider pre-treatment processes and influent organic matter concentrations when comparing the performance of specific DWTP processes.

7.2 Implications for Municipal Drinking Water Treatment Plants

Based on the results of the present study, the following recommendations are made for municipal drinking water providers:

- ATP analysis can provide an indication of the quantity of viable biomass within biofilters and can be used to monitor biofilter biomass development. For this reason, it may be a useful monitoring tool for municipal DWTP which employ biological filtration. ATP analysis is also simple and fast to perform, and requires very little specialized laboratory equipment (kits now available commercially). Filter media samples for ATP analysis can be collected from the top of operational biofilters, without disrupting plant production.
- During this study, the biofilters were operated at an EBCT of 38 minutes on average. This long EBCT is due to the fact that the DWTP was designed to produce a little more than twice what it did during the present study. Although significant NOM removal was reported through the biofilters in this study, attention should be paid to the changes in biofilter performance as the EBCT is shortened due to increased plant production.
- The performance of biofilters has been shown to be impacted by seasonal changes in raw water quality and temperature. Therefore, as climate change in North America continues to affect weather patterns, the continued monitoring of biofilter performance is important. The effect of extreme weather events on biofilter performance, and overall plant performance, should also be investigated.
- In the present study, SBC achieved significant removal of organic matter fractions in a DWTP. Therefore, when designing and upgrading DWTPs, SBC may be considered as an alternative to traditional coagulation-flocculation-sedimentation.

7.3 Recommendations for Future Research

Based on the study performed, the following recommendations are suggested for future research:

- SBC performance in terms of NOM removal at the HWTP was considerable throughout the present study. However, head-to-head studies comparing the efficiency of SBC, conventional coagulation, and enhanced coagulation strategies in drinking water applications have not been reported in the peer-reviewed literature. Such studies would be beneficial to identify the factors that contribute most significantly to NOM removal in each of these processes, and to determine the advantages and disadvantages of each. It will also be important to evaluate the differences in process performance when using different coagulants, such as aluminum sulfate and polyaluminum chloride, and when applying coagulant aids, such as polymers and silicate.

- ATP concentration is used as an indicator of the quantity of viable biomass within drinking water treatment biofilters, and a review of the published literature has found no relationship between biofilter performance and ATP concentration (as was the case in the present study). Therefore, further research should be performed to determine if this parameter is useful in terms of describing the quantity of biomass present in biofilters, or if it can somehow provide an indication of biofilter performance.
- LC-OCD provided valuable information relating to the removal of specific NOM fractions through DWTP processes. As this technique is relatively new, further investigation into the removal of specific NOM fractions, and their characteristics should be performed. In addition, further investigations should be performed to identify the biodegradable NOM fractions quantified by LC-OCD.
- Ammonia removal through biofiltration has been widely reported, however, no significant removal of ammonia was observed at the studied drinking water treatment plant. Hypotheses were developed to explain why ammonia removal was not observed, however, to gain a true understanding of this phenomenon, further investigation should be performed. Studies investigating the microbial community within the biofilters might help to determine if nitrifying bacteria are present within the biomass.
- Through the biofilters at the HWTP, significant NOM removal was obtained at an EBCT of 38 minutes. As this EBCT is much longer than what is typically used at full-scale DWTPs, studies should be performed to confirm the results from the present study at shorter biofilter EBCTs.
- Through the present study, good biofilter performance and average to above average biomass quantity and activity were reported even though nutrient limitations, as determined by CNP ratio, were observed in the biofilter feed. For this reason, further investigation into the types of nutrients required for biomass growth should be undertaken. Additionally, research should focus on identifying the nutrient forms which are most easily biodegradable, and those that should be used to calculate meaningful C:N:P ratios.

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Appendix A

Investigation into Biological Activity within a Sand-ballasted Clarification Process

Due to the considerable removal of DOC observed through SBC, investigations were undertaken to ensure that the DOC removal was solely due to coagulation-flocculation-sedimentation and not by biological processes. Biological processes could contribute to the DOC removal as a result of possible biofilm development on the microsand due to long HRT in the sand-ballasted clarification (SBC) unit and recycling of microsand. Therefore, samples of microsand were collected, and analyzed for adenosine triphosphate (ATP) to measure the quantity of viable microorganisms present. The microsand sample was taken after the hydrocyclone, prior to injection into the SBC process. The results from this analysis showed that there was no significant active biomass present on the surface of the microsand. As further support, biological processes are known to be impacted by cold temperatures; however, the performance of the SBC unit did not change seasonally (Rittman & McCarty, 2001).

Table A.1: Biomass quantity on microsand in SBC process

ATP sample (RLU)	Wet weight media (g)	Dry weight media (g)	Total ATP (ng ATP/g dry media)	Total ATP (ng ATP/cm^3 media)
61917	1.34	1.08	65	115*

RLU—relative light units.

*Converted to a volume basis using the average bulk density of microsand of 1.77 g/mL (I. Krüger, Inc., 2011)

Appendix B

Additional Information on Biomass Quantity and Activity of Biofilter Core Samples

In March 2013, core samples of the biofilters at the HWTP were taken. Two sets of core samples were taken, one from a biofilter prior to backwash, after 60 hours of operation, and the other from a biofilter after backwash. Analyses of the quantity of biomass, measured by ATP, and the activity of the biomass, measured by FDA, were undertaken. Media samples were taken at 15 cm intervals through the depth of the biofilter up to a depth of 122 cm. A sand sample from a depth of 168 cm was also taken. Results below indicate that the biomass quantity, quantified as ATP, decreased through the depth of the biofilter prior to backwash. After backwash, the quantity of biomass was relatively constant through the depth of the biofilter. Results of the biomass activity appear to demonstrate that the activity of the biomass decreased in non-backwashed biofilters through the depth of the biofilter. In the backwashed biofilters, the activity appears to increase initially, and then decreases through the depth.

Table B.1: Biomass quantity through the depth of a biofilter at the HWTP

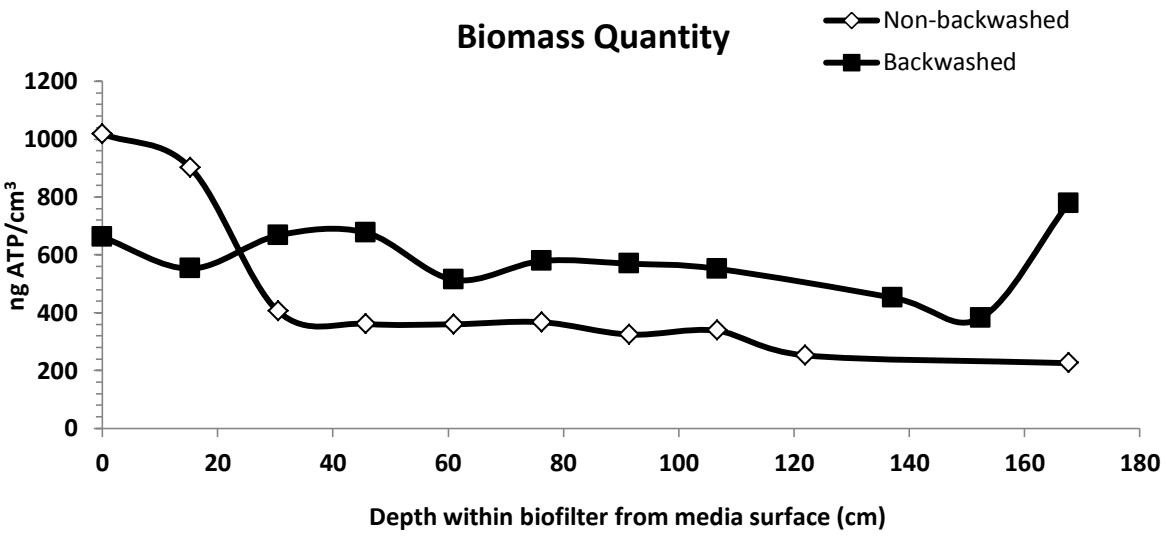
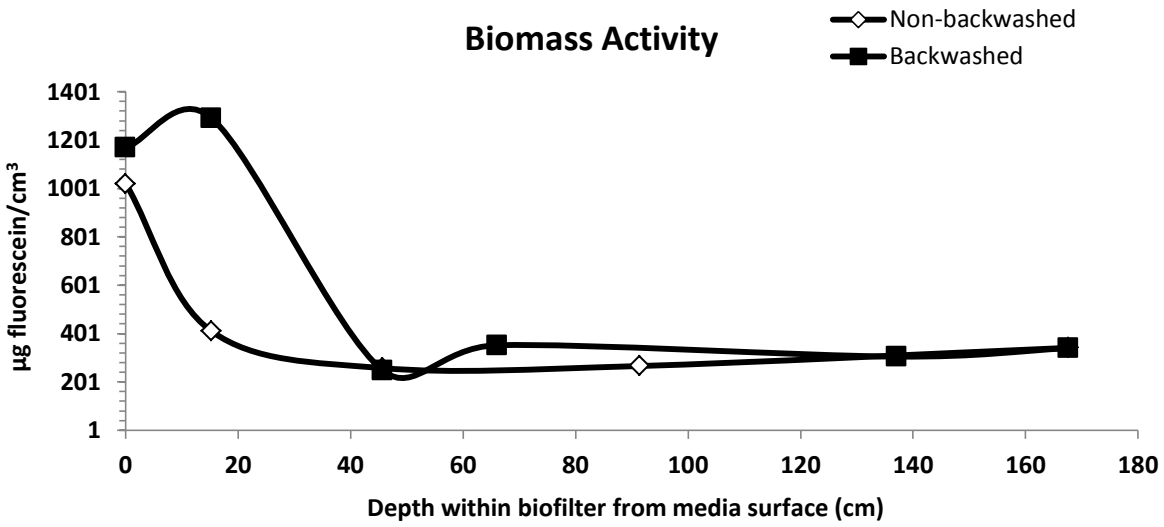


Table B.2: Biomass activity through the depth of a biofilter at the HWTP



Appendix C

Raw Laboratory Data

Table C.1: Raw temperature and pH data

Date	Temperature (°C)					pH				
	1	2	3	4	5	1	2	3	4	5
25-May-12	23	23	23	23	24	7.63	7.23	7.52	7.84	7.81
12-Jun-12	24	25	25	24	25	8.74	7.43	7.54	7.54	7.16
26-Jun-12	23	23	23	24	23	8.66	7.85	7.90	8.19	7.48
09-Jul-12	27	27	27	27	27	8.17	7.40	7.50	7.67	7.31
24-Jul-12	28	28	28	28	28	8.37	7.33	7.47	7.46	7.13
08-Aug-12	26	25	26	26	26	8.44	7.50	7.62	7.57	7.28
21-Aug-12	22	21	22	23	23	8.64	7.76	7.83	7.76	7.40
05-Sep-12	23	22	24	24	24	-	-	-	-	-
18-Sep-12	20	20	20	21	20	8.22	7.68	7.72	7.70	7.42
02-Oct-12	17	16	17	-	17	8.30	7.69	7.68	7.63	7.35
15-Oct-12	14	15	14	15	15	8.10	7.70	7.79	7.80	7.53
30-Oct-12	9	9	10	10	11	7.99	7.60	7.63	7.62	7.35
13-Nov-12	10	10	11	10	11	8.10	7.69	7.78	7.91	7.50
27-Nov-12	7	6	7	6	7	8.23	7.83	7.88	7.96	7.62
11-Dec-12	5	6	6	6	7	8.16	7.75	7.79	7.78	7.50
08-Jan-13	4	4	4	4	5	8.18	7.81	7.78	7.80	7.40
23-Jan-12	4	2	3	3	1	7.22	7.57	7.52	7.55	7.31
04-Feb-13	3	4	3	5	5	7.98	7.48	7.42	7.58	7.08
18-Feb-13	3	3	3	3	4	8.16	7.78	7.81	7.81	7.47
06-Mar-13	5	4	4	7	5	8.12	7.71	7.75	7.71	7.41
20-Mar-13	4	5	4	-	5	8.12	7.71	7.70	7.65	7.34

1-Raw, 2- SBC effluent, 3-Ozone effluent, 4-Biofilter effluent, 5-Chlorine effluent.

Table C.1: Raw temperature and pH data (con't)

Date	Temperature (°C)					pH				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
03-Apr-13	5	6	7	6	8	8.02	7.55	7.63	7.60	7.32
15-Apr-13	8	7	7	7	9	7.67	7.31	7.48	7.51	7.21
29-Apr-13	14	14	14	14	15	8.00	7.58	7.63	7.62	7.33
13-May-13	14	13	14	14	16	8.06	7.39	7.55	7.52	7.26
27-May-13	18	17	17	17	18	8.29	7.66	7.72	7.69	7.39
12-Jun-13	21	20	20	20	21	8.11	7.53	7.66	7.56	7.36
24-Jun-13	26	26	26	26	27	8.44	7.64	7.72	7.60	7.51
08-Jul-13	25	25	25	25	25	8.20	7.56	7.64	7.56	7.43
22-Jul-13	26	25	25	27	26	8.34	7.55	7.66	7.55	7.40
29-Jul-13	21	21	21	22	22	8.31	7.63	7.66	7.59	7.40

1-Raw, 2- SBC effluent, 3-Ozone effluent, 4-Biofilter effluent, 5-Chlorine effluent.

Table C.2: Raw conductivity and UVA_{254} data

Date	Conductivity (µs/cm)					UVA_{254} (cm^{-1})*				
	1	2	3	4	5	1	2	3	4	5
25-May-12	812	830	830	840	843	0.1391	0.0779	0.0518	0.0446	0.0336
12-Jun-12	760	751	752	752	753	0.1595	0.0980	0.0552	0.0563	0.0459
26-Jun-12	754	771	766	757	760	0.1531	0.0938	0.0590	0.0579	0.0449
09-Jul-12	751	772	775	777	787	0.1506	0.0846	0.0454	0.0410	0.0033
24-Jul-12	768	784	801	811	825	0.1521	0.0882	0.0527	0.0481	0.0430
08-Aug-12	742	769	773	784	803	0.1469	0.0878	0.0518	0.0457	0.0424
21-Aug-12	780	806	805	809	824	0.1334	0.0797	0.0428	0.0398	0.0354
05-Sep-12	775	785	790	797	815	0.1425	0.0890	0.0520	0.0501	0.0536
18-Sep-12	846	864	868	874	903	0.1211	0.0808	0.0475	0.0448	0.0372
02-Oct-12	810	824	819	819	824	0.1188	0.0818	0.0491	0.0456	0.0398
15-Oct-12	836	863	858	860	856	0.1173	0.0783	0.0522	0.0484	0.0402
30-Oct-12	648	654	656	656	652	0.1713	0.1012	0.0644	0.0600	0.0459
13-Nov-12	940	960	960	950	950	0.1680	0.1030	0.0765	0.0750	0.0580
27-Nov-12	820	833	831	830	831	0.1555	0.1075	0.0770	0.0760	0.0620
11-Dec-12	808	828	832	828	822	0.1740	0.1030	0.0780	0.0785	0.0670
08-Jan-13	846	855	855	854	852	0.1430	0.0930	0.0630	0.0650	0.0545
23-Jan-12	855	861	853	848	833	0.1507	0.0787	0.0527	0.0510	0.0380
04-Feb-13	510	516	513	510	504	0.1963	0.0873	0.0597	0.0607	0.0500
18-Feb-13	888	911	914	915	907	0.1330	0.0787	0.0577	0.0560	0.0450
06-Mar-13	750	760	760	765	780	0.1339	0.0772	0.0529	0.0503	0.0431
20-Mar-13	522	534	516	517	505	0.1610	0.0815	0.0578	0.0565	0.0445

* Average of triplicate measurements reported.

1-Raw, 2- SBC effluent, 3-Ozone effluent, 4-Biofilter effluent, 5-Chlorine effluent.

Table C.2: Raw conductivity and UVA_{254} data (con't)

Date	Conductivity (µs/cm)					UVA_{254} (cm^{-1})*				
	1	2	3	4	5	1	2	3	4	5
03-Apr-13	498	507	506	507	507	0.1345	0.0708	0.0457	0.0433	0.0362
15-Apr-13	374	386	390	392	398	0.1677	0.0834	0.0525	0.0498	0.0424
29-Apr-13	542	561	564	568	579	0.1503	0.0842	0.0553	0.0536	0.0407
13-May-13	704	712	715	713	732	0.1537	0.0835	0.0579	0.0580	0.0456
27-May-13	612	635	638	638	641	0.1828	0.0968	0.0691	0.0653	0.0484
12-Jun-13	674	697	705	706	716	0.1682	0.0904	0.0632	0.0557	0.0422
24-Jun-13	722	751	751	755	753	0.1698	0.0864	0.0649	0.0533	0.0441
08-Jul-13	643	658	655	651	644	0.2026	0.1115	0.0735	0.0675	0.0504
22-Jul-13	651	676	678	680	690	0.1830	0.1006	0.0593	0.0513	0.0332
29-Jul-13	726	749	758	767	784	0.1792	0.1034	0.0594	0.0570	0.0425

* Average of triplicate measurements reported.

1-Raw, 2- SBC effluent, 3-Ozone effluent, 4-Biofilter effluent, 5-Chlorine effluent.

Table C.3: Raw TOC data

Date	TOC (mg/L)				
	Raw	SBC effluent	Ozone effluent	Biofilter effluent	Chlorine effluent
25-May-12	6.09	4.26	4.09	3.28	3.10
12-Jun-12	5.93	4.25	4.57	3.81	3.47
26-Jun-12	6.13	4.61	4.49	3.69	3.61
09-Jul-12	5.88	4.16	4.04	3.25	3.02
24-Jul-12	6.40	4.35	4.28	3.38	3.28
08-Aug-12	6.48	4.52	4.05	2.88	3.17
21-Aug-12	6.06	4.20	4.02	3.21	2.99
05-Sep-12	6.06	4.47	4.30	3.44	3.24
18-Sep-12	5.34	4.17	4.08	3.21	3.23
02-Oct-12	5.53	4.40	4.22	3.57	3.13
15-Oct-12	5.43	4.32	4.24	3.28	3.39
30-Oct-12	6.65	4.67	4.52	3.70	3.47
13-Nov-12	6.25	4.77	4.74	4.26	4.04
27-Nov-12	6.09	4.99	4.79	4.16	4.03
11-Dec-12	6.00	4.48	4.36	3.94	3.70
23-Jan-13	6.44	4.55	4.33	3.73	3.52
04-Feb-13	7.42	4.44	4.39	3.80	3.57
18-Feb-13	5.51	4.10	4.08	3.56	3.47
06-Mar-13	5.42	4.01	3.97	3.44	3.25
20-Mar-13	6.15	4.14	3.95	3.48	3.26
03-Apr-13	5.57	3.69	3.64	3.07	2.89
15-Apr-13	6.36	3.99	2.91	3.09	2.88
29-Apr-13	5.86	4.01	3.99	3.00	3.17
13-May-13	5.83	4.03	3.93	3.19	3.07
27-May-13	7.16	4.36	4.29	3.54	3.25
12-Jun-13	6.17	4.25	4.27	3.34	2.99
26-Jun-13	6.48	4.21	4.14	3.35	3.02
08-Jul-13	6.63	4.76	5.38	3.71	3.27
22-Jul-13	6.53	4.52	4.26	3.39	3.22
29-Jul-13	6.85	4.59	4.24	3.62	3.21
Mean	6.16	4.34	4.22	3.48	3.30
STD	0.50	0.28	0.41	0.32	0.29

Table C.4: Raw DOC data

Date	DOC (mg/L)				
	Raw	SBC effluent	Ozone effluent	Biofilter effluent	Chlorine effluent
25-May-12	5.84	3.93	3.79	3.30	3.10
12-Jun-12	5.77	4.43	4.37	3.64	3.32
26-Jun-12	6.11	4.49	4.28	3.73	3.52
09-Jul-12	5.89	4.03	3.77	3.29	2.99
24-Jul-12	6.15	4.19	3.99	3.39	3.24
08-Aug-12	6.23	4.27	3.99	3.42	3.18
21-Aug-12	5.76	3.94	3.86	3.18	3.05
05-Sep-12	6.07	4.38	4.14	3.51	3.23
18-Sep-12	5.35	4.04	3.83	3.24	3.18
02-Oct-12	5.47	4.19	3.98	3.50	3.23
15-Oct-12	5.54	4.11	3.99	3.41	3.22
30-Oct-12	6.43	4.42	4.10	3.74	3.48
13-Nov-12	6.17	4.49	4.50	4.22	4.00
27-Nov-12	6.00	4.75	4.55	4.23	4.08
11-Dec-12	6.00	4.15	4.40	3.86	3.78
23-Jan-13	6.24	4.22	4.27	3.73	3.53
04-Feb-13	6.88	4.13	3.98	3.72	3.53
18-Feb-13	5.36	3.96	3.59	3.53	3.51
06-Mar-13	5.13	3.77	3.62	3.45	3.09
20-Mar-13	5.92	3.75	3.66	3.47	3.23
03-Apr-13	5.39	3.51	3.40	3.09	2.87
15-Apr-13	5.94	3.74	3.47	3.05	2.83
29-Apr-13	5.44	3.92	3.77	3.32	3.07
13-May-13	5.72	3.77	3.69	3.23	3.06
27-May-13	6.40	4.18	3.97	3.38	3.16
12-Jun-13	5.99	3.93	3.89	3.26	3.03
24-Jun-13	6.24	3.99	4.03	3.47	3.02
08-Jul-13	6.77	4.58	4.29	3.75	3.43
22-Jul-13	6.34	4.41	4.20	3.47	3.11
29-Jul-13	6.61	4.22	4.10	3.59	3.24
Mean	5.97	4.13	3.98	3.51	3.28
STD	0.43	0.29	0.30	0.28	0.30

Table C.5: Raw total nitrogen and nitrate data

Date	Total nitrogen (mg N/L)					Nitrate (mg N-NO_3/L)				
	1	2	3	4	5	1	2	3	4	5
25-May-12	3.2	3.2	4.2	2.7	2.6	1.9	1.9	1.8	2.6	2.6
12-Jun-12	5.2	4.1	2.5	2.0	5.9	3.0	2.9	3.3	3.5	4.8
26-Jun-12	5.0	3.9	1.8	5.1	1.7	2.4	2.3	2.5	3.1	3.0
09-Jul-12	3.9	2.2	2.0	2.5	4.0	1.9	2.2	2.4	2.3	3.1
24-Jul-12	<0.5	5.5	<0.5	0.9	<0.5	2.1	1.6	2.0	2.3	3.4
08-Aug-12	-	-	-	-	-	1.4	1.6	1.5	1.8	2.8
21-Aug-12	4.0	7.4	1.4	2.7	<0.5	1.9	2.1	2.3	2.2	3.4
05-Sep-12	6.0	3.6	5.0	6.7	3.1	1.7	1.7	2.0	1.9	3.4
18-Sep-12	1.4	3.4	0.8	2.8	0.5	2.6	2.7	2.6	3.2	4.0
02-Oct-12	5.0	4.7	5.7	4.0	3.8	3.1	2.9	3.3	3.4	4.8
15-Oct-12	4.7	4.4	3.9	4.5	3.4	3.6	3.4	3.7	3.3	4.4
30-Oct-12	5.7	3.6	3.3	3.2	5.2	2.6	2.9	3.0	3.5	3.8
13-Nov-12	8.8	5.3	6.2	6.5	6.9	4.2	4.7	4.6	5.0	6.1
27-Nov-12	-	-	-	-	-	4.5	4.3	4.1	4.5	5.0
11-Dec-12	-	-	-	-	-	4.7	4.6	4.3	5.2	6.1
08-Jan-13	6.8	6.6	7.9	6.6	8.6	4.8	3.9	4.5	5.2	6.2
23-Jan-12	7.3	7.8	6.9	7.4	7.5	5.5	5.0	5.2	5.7	6.2
04-Feb-13	-	-	-	-	-	4.5	4.3	4.6	5.0	5.7
18-Feb-13	6.7	6.9	6.8	6.2	8.9	4.9	4.1	4.1	4.6	5.8
06-Mar-13	6.7	8.7	9.5	5.7	5.6	4.0	4.1	4.1	4.7	5.9
20-Mar-13	4.7	3.8	3.5	3.7	8.9	3.2	3.2	3.6	3.8	4.8

1-Raw, 2- SBC effluent, 3-Ozone effluent, 4-Biofilter effluent, 5-Chlorine effluent.

Table C.5: Raw total nitrogen and nitrate data (con`t)

Date	Total nitrogen (mg N/L)					Nitrate (mg N-NO_3/L)				
	1	2	3	4	5	1	2	3	4	5
03-Apr-13	-	-	-	-	-	4.7	4.5	4.9	4.7	5.8
15-Apr-13	-	-	-	-	-	3.1	3.7	3.8	4.4	5.0
29-Apr-13	2.8	3.4	3.9	3.5	3.6	3.4	3.3	3.5	3.8	4.9
13-May-13	3.3	2.9	3.2	3.0	3.2	2.9	2.9	2.8	3.2	4.1
27-May-13	4.2	5.8	5.9	6.0	6.1	3.9	4.3	4.6	4.8	5.6
12-Jun-13	-	-	-	-	-	3.1	2.9	2.2	3.4	4.3
24-Jun-13	-	-	-	-	-	2.3	2.5	2.5	3.0	3.9
08-Jul-13	3.5	3.7	4.3	2.9	3.8	2.7	3.2	2.9	3.6	4.3
22-Jul-13	2.6	2.6	2.8	3.0	3.3	1.6	1.6	1.8	2.2	3.1
29-Jul-13	3.2	2.8	3.4	4.6	3.8	2.5	2.4	2.6	3.3	4.6

1-Raw, 2- SBC effluent, 3-Ozone effluent, 4-Biofilter effluent, 5-Chlorine effluent.

Table C.6: Raw ammonia data

Date	Ammonia (mg N-NH_3/L)				
	Raw	SBC effluent	Ozone effluent	Biofilter effluent	Chlorine effluent
25-May-12	0.06	0.04	0.06	0.07	<0.02
12-Jun-12	-	-	-	-	-
26-Jun-12	<0.02	0.02	0.03	<0.02	<0.02
09-Jul-12	0.08	0.09	0.07	0.19	<0.02
24-Jul-12	-	-	-	-	-
08-Aug-12	-	-	-	-	-
21-Aug-12	<0.02	0.12	0.02	<0.02	<0.02
05-Sep-12	0.05	0.04	0.03	<0.02	<0.02
18-Sep-12	0.05	0.04	0.04	<0.02	<0.02
02-Oct-12	0.02	<0.02	0.02	<0.02	<0.02
15-Oct-12	<0.02	<0.02	<0.02	<0.02	<0.02
30-Oct-12	0.11	0.09	0.11	0.11	<0.02
13-Nov-12	0.16	0.16	0.16	0.15	<0.02
27-Nov-12	0.15	0.15	0.13	0.16	0.04
11-Dec-12	0.27	0.27	0.31	0.27	0.02
8-Jan-13	0.58	0.63	0.63	0.69	<0.02
23-Jan-13	-	-	-	-	-
04-Feb-13	0.19	0.18	0.21	0.21	<0.02
18-Feb-13	-	-	-	-	-
06-Mar-13	0.36	0.40	0.38	0.38	<0.02
20-Mar-13	0.17	0.16	0.20	0.19	<0.02
03-Apr-13	0.03	0.04	0.05	0.06	<0.02
15-Apr-13	0.07	0.06	0.09	0.10	0.02
29-Apr-13	-	-	-	-	-
13-May-13	0.02	0.02	0.04	0.04	<0.02
27-May-13	0.06	0.07	0.09	0.06	<0.02
12-Jun-13	0.07	0.04	0.09	<0.02	<0.02
24-Jun-13	0.03	0.07	0.06	<0.02	<0.02
08-Jul-13	0.04	0.03	0.06	<0.02	<0.02
22-Jul-13	0.05	0.02	<0.02	<0.02	0.31
29-Jul-13	0.04	0.05	0.05	<0.02	<0.02

Table C.7: Raw ATP and FDA data

Date	Filter #	Filter Flow Rate (L/s)	ATP (ng ATP/cm^3)	FDA (µg fluorescein/cm^3)
26-Jun-12	7	-	1439	763
09-Jul-12	7	-	1028	632
24-Jul-12	7	-	724	555
08-Aug-12	3	-	705	340
21-Aug-12	7	82	1132	339
05-Sep-12	7	67	1786	557
18-Sep-12	7	73	2037	540
02-Oct-12	7	77	1310	531
15-Oct-12	3	67	877	314
30-Oct-12	7	61.77	1067	542
13-Nov-12	7	67.5	1129	-
27-Nov-12	7	58.54	1413	472
11-Dec-12	7	61	1736	540
08-Jan-13	7	57	-	472
23-Jan-13	7	50	1102	468
04-Feb-13	5	62	945	494
18-Feb-13	7	62.5	1597	522
06-Mar-13	7	53.3	1096	421
20-Mar-13	5	-	1018	554
03-Apr-13	7	58.3	1584	571
15-Apr-13	3	49	817	237
29-Apr-13	7	66.7	1201	836
13-May-13	7	77.5	979	713
27-May-13	7	68.3	1530	713
12-Jun-13	7	68.3	1830	710
26-Jun-13	7	73.3	1114	527
08-Jul-13	7	67.5	1417	619
22-Jul-13	7	77.5	1583	648
29-Jul-13	7	77.5	1318	726

Table C.8: Raw orthophosphate data

Date	Orthophosphate (mg PO_4^{3-}/L)				
	Raw	SBC effluent	Ozone effluent	Biofilter effluent	Chlorine effluent
13-Nov-12	0.01	0.003	0.0031	<0.0030	0.0033
08-Jan-13	0.0073	<0.0030	<0.0030	<0.0030	0.0031
04-Feb-13	0.0335	0.004	0.0046	0.0035	-
18-Feb-13	0.0163	<0.0030	0.0033	0.0033	-
06-Mar-13	0.0088	<0.0030	<0.0030	<0.0030	-
20-Mar-13	0.0219	<0.0030	<0.0030	<0.0030	-
03-Apr-13	0.0228	<0.0030	<0.0030	<0.0030	-
15-Apr-13	0.0357	<0.0030	<0.0030	<0.0030	-
29-Apr-13	0.0042	<0.0030	<0.0030	<0.0030	-
13-May-13	0.0037	<0.0030	<0.0030	<0.0030	-
27-May-13	0.0138	0.0037	<0.0030	<0.0030	-
12-Jun-13	0.0143	<0.0030	<0.0030	<0.0030	-
26-Jun-13	0.0045	<0.0030	<0.0030	<0.0030	-
08-Jul-13	0.0197	<0.0030	<0.0030	<0.0030	-
22-Jul-13	0.0076	<0.0030	<0.0030	<0.0030	-
29-Jul-13	0.0046	<0.0030	<0.0030	<0.0030	-

Table C.9: Raw total phosphorus data

Date	Total phosphorus (mg P/L)			
	Raw	SBC effluent	Ozone effluent	Biofilter effluent
06-Mar-13	0.0294	0.006	0.0071	0.0045
20-Mar-13	0.0322	0.0051	0.0143	<0.0030
03-Apr-13	0.0451	0.0075	0.0094	0.0031
15-Apr-13	0.088	0.0118	0.008	0.0038
29-Apr-13	0.0436	0.0101	0.0312	0.0052
13-May-13	0.0337	0.0079	0.0072	0.0035
27-May-13	0.0569	0.0092	0.0134	0.0037
12-Jun-13	0.0463	0.0099	0.0121	0.0042
26-Jun-13	0.0209	<0.0030	<0.0030	0.0081
08-Jul-13	0.0584	0.0107	0.045	<0.0030
22-Jul-13	0.0513	0.0106	0.0113	0.0045
29-Jul-13	0.037	0.0183	0.0239	0.0104

Table C.10: Raw total dissolved phosphorus data

Date	Total dissolved phosphorus (mg P/L)			
	Raw	SBC effluent	Ozone effluent	Biofilter effluent
03-Apr-13	0.0328	0.006	0.0048	<0.0030
15-Apr-13	0.0428	<0.0030	<0.0031	0.0389
29-Apr-13	0.0115	0.0055	0.006	0.0046
13-May-13	0.0129	0.0058	0.0052	0.0039
27-May-13	0.0243	0.0039	0.0033	<0.0030
12-Jun-13	0.0248	0.0043	0.0068	0.0043
26-Jun-13	0.0154	<0.0030	<0.0030	0.026
08-Jul-13	0.0285	0.0043	<0.0030	<0.0030
22-Jul-13	0.0204	0.0069	0.0063	0.004
29-Jul-13	0.0331	0.0161	0.0078	0.0063

Table C.11: Raw AOC data

Date	AOC (µg/L)		
	SBC effluent	Ozone effluent	Biofilter effluent
22-May-12	129	384	96
12-Jun-12	59	225	77
26-Jun-12	59	245	72
09-Jul-12	51	260	104
24-Jul-12	39	266	203
08-Aug-12	58	300	169
21-Aug-12	69	373	157
05-Sep-12	53	355	124
18-Sep-12	96	295	123
02-Oct-12	70	358	167
15-Oct-12	76	331	170
30-Oct-12	80	345	170
13-Nov-12	80	310	248
08-Jan-13	83	273	133
23-Jan-13	173	380	247
04-Feb-13	122	383	261
18-Feb-13	108	309	225
06-Mar-13	80	334	194
03-Apr-13	69	287	138
15-Apr-13	83	385	177
29-Apr-13	112	418	189
13-May-13	166	369	187
27-May-13	125	383	197
12-Jun-13	73	338	132
26-Jun-13	65	244	82
08-Jul-13	61	292	88
22-Jul-13	96	417	155
29-Jul-13	141	302	233

Table C.12: Raw LC-OCD biopolymer data

Date	LC-OCD biopolymer concentration (mg/L)				
	Raw	SBC effluent	Ozone effluent	Biofilter effluent	Chlorine effluent
25-May-12	0.96	0.47	0.44	0.32	0.34
12-Jun-12	0.73	0.35	0.31	0.22	0.22
26-Jun-12	0.77	0.37	0.34	0.21	0.28
09-Jul-12	0.67	0.32	0.28	0.20	0.22
24-Jul-12	0.63	0.31	0.31	0.18	0.25
30-Oct-12	0.42	0.20	0.18	0.13	0.14
13-Nov-12	0.41	0.19	0.18	0.14	0.16
27-Nov-12	0.51	0.24	0.25	0.23	0.24
11-Dec-12	0.42	0.17	0.19	0.14	0.16
08-Jan-13	0.33	0.16	0.17	0.15	0.17
23-Jan-13	0.28	0.11	0.11	0.10	0.10
04-Feb-13	0.44	0.14	0.14	0.13	0.14
18-Feb-13	0.27	0.12	0.12	0.11	0.11
20-Mar-13	0.36	0.14	0.14	0.14	0.15
15-Apr-13	0.35	0.15	0.14	0.10	0.12
29-Apr-13	0.35	0.19	0.19	0.13	0.13
13-May-13	0.48	0.24	0.23	0.17	0.16
27-May-13	0.43	0.24	0.22	0.14	0.17
12-Jun-13	0.38	0.21	0.18	0.10	0.12
26-Jun-13	0.55	0.24	0.22	0.10	0.12
08-Jul-13	0.34	0.19	0.17	0.08	0.08
22-Jul-13	0.44	0.21	0.19	0.06	0.08
29-Jul-13	0.47	0.25	0.24	0.13	0.14

Table C.13: Raw LC-OCD humic substance data

Date	LC-OCD humic substance concentration (mg/L)				
	Raw	SBC effluent	Ozone effluent	Biofilter effluent	Chlorine effluent
25-May-12	3.32	1.80	1.91	1.62	1.55
12-Jun-12	3.20	2.06	1.79	1.71	1.67
26-Jun-12	3.22	1.68	1.61	1.48	1.40
09-Jul-12	3.12	2.23	2.08	2.07	1.81
24-Jul-12	3.32	1.80	1.91	1.62	1.55
30-Oct-12	3.48	2.26	2.05	1.79	1.76
13-Nov-12	3.40	2.14	2.03	2.09	2.03
27-Nov-12	3.17	2.34	2.26	2.16	2.10
11-Dec-12	3.72	2.32	2.17	2.17	2.05
08-Jan-13	3.25	2.04	1.94	1.95	1.76
23-Jan-13	3.54	1.88	1.79	1.66	1.58
04-Feb-13	4.02	1.87	1.96	1.89	1.83
18-Feb-13	2.80	1.78	1.73	1.61	1.52
20-Mar-13	3.46	1.89	1.76	1.84	1.70
15-Apr-13	3.59	1.85	1.73	1.57	1.53
29-Apr-13	3.00	1.97	1.84	1.77	1.66
13-May-13	3.05	1.74	1.70	1.60	1.47
27-May-13	3.80	2.14	2.10	1.99	1.88
12-Jun-13	3.44	2.03	2.01	1.75	1.66
26-Jun-13	3.59	1.84	1.88	1.62	1.69
08-Jul-13	4.03	2.31	2.03	1.96	1.77
22-Jul-13	3.52	2.08	2.02	1.73	1.70
29-Jul-13	3.66	2.26	2.03	1.93	1.75

Table C.14: Raw LC-OCD building blocks data

Date	LC-OCD building blocks concentration (mg/L)				
	Raw	SBC effluent	Ozone effluent	Biofilter effluent	Chlorine effluent
25-May-12	0.87	0.75	0.65	0.66	0.60
12-Jun-12	0.86	0.88	0.70	0.69	0.63
26-Jun-12	1.00	0.78	1.01	0.81	0.89
09-Jul-12	1.01	0.95	0.97	0.81	0.78
24-Jul-12	0.93	0.37	0.43	0.23	0.42
30-Oct-12	1.01	0.75	0.82	0.77	0.61
13-Nov-12	0.97	0.88	0.94	0.75	0.71
27-Nov-12	0.96	0.93	0.87	0.85	0.77
11-Dec-12	0.80	0.79	0.83	0.78	0.83
08-Jan-13	0.66	0.86	0.82	0.74	0.80
23-Jan-13	0.88	0.92	0.93	0.91	0.86
04-Feb-13	0.83	0.85	0.68	0.68	0.63
18-Feb-13	0.82	0.74	0.69	0.73	0.73
20-Mar-13	0.59	0.56	0.55	0.41	0.41
15-Apr-13	0.44	0.47	0.42	0.40	0.36
29-Apr-13	0.71	0.51	0.46	0.44	0.45
13-May-13	0.68	0.61	0.60	0.53	0.57
27-May-13	0.64	0.55	0.57	0.47	0.50
12-Jun-13	0.55	0.57	0.52	0.50	0.45
26-Jun-13	0.70	0.63	0.54	0.51	0.40
08-Jul-13	0.76	0.62	0.68	0.54	0.51
22-Jul-13	0.87	0.64	0.61	0.51	0.39
29-Jul-13	0.66	0.61	0.70	0.57	0.51

Table C.15: Raw LC-OCD LMW acids and humics data

Date	LC-OCD LMW acids and humics concentration (mg/L)				
	Raw	SBC effluent	Ozone effluent	Biofilter effluent	Chlorine effluent
25-May-12	0.18	0.15	0.23	0.16	0.16
12-Jun-12	0.17	0.16	0.24	0.15	0.15
26-Jun-12	0.20	0.18	0.26	0.16	0.01
09-Jul-12	0.19	0.15	0.29	0.20	0.16
24-Jul-12	0.22	0.21	0.28	0.17	0.17
30-Oct-12	0.16	0.14	0.24	0.14	0.16
13-Nov-12	0.16	0.14	0.19	0.14	0.14
27-Nov-12	0.15	0.13	0.20	0.15	0.14
11-Dec-12	0.18	0.14	0.18	0.16	0.14
08-Jan-13	0.14	0.13	0.18	0.14	0.14
23-Jan-13	0.16	0.14	0.20	0.15	0.14
04-Feb-13	0.17	0.16	0.20	0.17	0.16
18-Feb-13	0.12	0.09	0.12	0.10	0.10
20-Mar-13	0.09	0.07	0.08	0.07	0.05
15-Apr-13	0.09	0.08	0.09	0.07	0.05
29-Apr-13	0.12	0.10	0.10	0.06	0.08
13-May-13	0.12	0.10	0.12	0.08	0.08
27-May-13	0.13	0.11	0.14	0.09	0.12
12-Jun-13	0.12	0.11	0.17	0.08	0.09
26-Jun-13	0.12	0.09	0.13	0.09	0.09
08-Jul-13	0.12	0.11	0.12	0.09	0.10
22-Jul-13	0.15	0.12	0.19	0.10	0.13
29-Jul-13	0.18	0.12	0.14	0.11	0.10

LMW – low molecular weight

Table C.16: Raw LC-OCD LMW neutrals data

Date	LC-OCD LMW neutrals concentration (mg/L)				
	Raw	SBC effluent	Ozone effluent	Biofilter effluent	Chlorine effluent
25-May-12	0.67	0.60	0.60	0.47	0.53
12-Jun-12	0.54	0.60	0.52	0.42	0.48
26-Jun-12	0.55	0.60	0.57	0.46	0.52
09-Jul-12	0.66	0.51	0.50	0.45	0.46
24-Jul-12	0.67	0.65	0.61	0.51	0.58
30-Oct-12	0.55	0.53	0.53	0.43	0.42
13-Nov-12	0.57	0.57	0.54	0.49	0.48
27-Nov-12	0.61	0.51	0.54	0.47	0.47
11-Dec-12	0.88	0.53	0.52	0.44	0.46
08-Jan-13	0.54	0.52	0.51	0.46	0.48
23-Jan-13	0.53	0.47	0.43	0.40	0.41
04-Feb-13	0.54	0.43	0.44	0.38	0.37
18-Feb-13	0.58	0.46	0.50	0.40	0.45
20-Mar-13	0.40	0.37	0.37	0.31	0.41
15-Apr-13	0.44	0.37	0.36	0.27	0.29
29-Apr-13	0.47	0.43	0.37	0.30	0.40
13-May-13	0.56	0.51	0.51	0.63	0.36
27-May-13	0.53	0.43	0.47	0.40	0.45
12-Jun-13	0.50	0.47	0.40	0.39	0.37
26-Jun-13	1.15	0.50	0.47	0.39	0.46
08-Jul-13	0.51	0.46	0.45	0.37	0.37
22-Jul-13	0.65	0.54	0.55	0.39	0.30
29-Jul-13	0.55	0.51	0.49	0.43	0.38

LMW – low molecular weight

Appendix D

Holmedale Water Treatment Plant Flow Rate and Coagulant Dose

Table D.1: HWTP Flow Rate and Coagulant Dose

Date	Raw Water Flows (ML/d)			Coagulant Dosage (mg/L)
	Minimum	Average	Maximum	Average
May-12	31.79	43.92	56.25	40.69
Jun-12	35.77	45.47	57.6	40.09
Jul-12	38.89	52.81	62.15	43.17
Aug-12	38.14	44.24	51.32	34.79
Sep-12	34.59	40.69	47.78	28.57
Oct-12	33.45	36.69	41.39	25.48
Nov-12	32.25	35.89	40.99	25.56
Dec-12	30.96	34.69	37.83	25.89
Jan-13	29.40	35.21	40.00	30.44
Feb-13	25.95	33.94	38.07	28.23
Mar-13	29.51	33.23	38.03	26.12
Apr-13	29.29	33.74	39.27	27.65
May-13	24.38	39.19	50.56	38.08
Jun-13	34.46	39.69	50.09	41.28
Jul-13	35.31	40.98	51.80	40.91